

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

_____)	
VENTANA MEDICAL SYSTEMS, INC.,)	
)	
Plaintiff,)	
v.)	C.A. No. 05-CV-10614-GAO
)	
VISION BIOSYSTEMS INC.,)	
)	
Defendant.)	
_____)	

**DECLARATION OF JOHN R. HUTCHINS
IN SUPPORT OF VISION BIOSYSTEMS INC.'s
MOTION FOR SUMMARY JUDGMENT OF NON-INFRINGEMENT**

I, John R. Hutchins, declare as follows:

1. I am a member of the law firm of Kenyon & Kenyon LLP, counsel to Vision BioSystems Inc. (“Vision”) in this action. I make this declaration in support of *Vision’s Motion For Summary Judgment Of Non-Infringement*. The following matters are true of my own personal knowledge.

2. Attached as **Exhibit 1** hereto is a true and correct copy of U.S. Patent No. 6,352,861 issued to Copeland et al.

3. Attached as **Exhibit 2** hereto is a true and correct copy of the Preliminary Amendment dated August 5, 1997 contained within the prosecution file of U.S. Patent No. 6,352,861.

4. Attached as **Exhibit 3** hereto is a true and correct copy of the Office Action dated May 10, 2001 contained within the prosecution file of U.S. Patent No. 6,352,861.

5. Attached as **Exhibit 4** hereto is a true and correct copy of the Information Disclosure Statement dated May 10, 2001 contained within the prosecution file of U.S. Patent No. 6,352,861.

6. Attached as **Exhibit 5** hereto is a true and correct copy of the Amendment dated June 26, 2001 contained within the prosecution file of U.S. Patent No. 6,352,861.

7. Attached as **Exhibit 6** hereto is a true and correct copy of the Office Action dated August 13, 2001 contained within the prosecution file of U.S. Patent No. 6,352,861.

8. Attached as **Exhibit 7** hereto is a true and correct copy of the Amendment dated August 24, 2001 contained within the prosecution file of U.S. Patent No. 6,352,861.

9. Attached as **Exhibit 8** hereto is a true and correct copy of an article written by Erwin Stark, Ditmar Faltinat, and Ruediger Von der Fecht entitled *An Automated Device for*

Immunocytochemistry published in the Journal of Immunological Methods in 1988, and referenced in the prosecution file of U.S. Patent No. 6,352,861.

10. Attached as **Exhibit 9** hereto is a true and correct copy of U.S. Patent No. 4,346,056 issued to Sakurada, referenced in the prosecution file of U.S. Patent No. 6,352,861.

11. Attached as **Exhibit 10** hereto is a true and correct copy of U.S. Patent No. 5,439,645 issued to Saralegui et al., referenced in the prosecution file of U.S. Patent No. 6,352,861.

12. Attached as **Exhibit 11** hereto is a true and correct copy of the Preliminary Amendment dated January 22, 2002 contained within the prosecution file of U.S. Patent No. 6,943,029.

13. Attached as **Exhibit 12** hereto is a true and correct copy of the Office Action dated October 30, 2002 contained within the prosecution file of U.S. Patent No. 6,943,029.

14. Attached as **Exhibit 13** hereto is a true and correct copy of the Office Action dated July 29, 2002 contained within the prosecution file of U.S. Patent No. 6,943,029.

15. Attached as **Exhibit 14** hereto is a true and correct copy of the Office Action dated April 22, 2004 contained within the prosecution file of U.S. Patent No. 6,943,029.

16. Attached as **Exhibit 15** hereto is a document displaying a side-by-side comparison of the allowed Claim 1 of U.S. Patent No. 6,943,029 and the rejected Claim 73 contained within the prosecution file of U.S. Patent No. 6,943,029.

17. Attached as **Exhibit 16** hereto is a true and correct copy of the Preliminary Amendment dated November 17, 2004 contained within the prosecution file of continuation application 10/991,050.

18. Attached as **Exhibit 17** hereto is a true and correct copy of U.S. Patent No. 6,943,029 issued to Copeland et al.

19. Attached as **Exhibit 18** hereto is a true and correct copy of U.S. Patent No. 5,235,167 issued to Dvorkis et al.

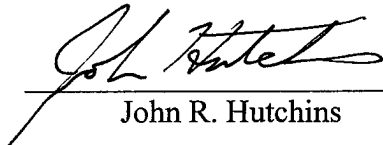
20. Attached as **Exhibit 19** hereto is a true and correct copy of *Ventana's Responses to Vision's Second Set of Interrogatories, Nos. 13 to 16* in Civil Action No. 05-CV-10614-GAO, dated September 19, 2005.

21. Attached as **Exhibit 20** hereto is a true and correct copy of U.S. Patent No. 5,646,046 issued to Fischer et al.

22. Attached as **Exhibit 21** hereto is a true and correct copy of the Second Preliminary Amendment dated March 8, 2005 contained within the prosecution file of continuation application 10/991,050.

23. Attached as **Exhibit 22** hereto is a true and correct copy of Ventana's Response to Interrogatory No. 1, contained within *Ventana's Responses to Vision's First Set of Interrogatories, Nos. 1 to 12* in Civil Action No. 05-CV-10614-GAO, dated August 15, 2005.

I declare under penalty of perjury that the foregoing is true and correct. Executed on June 18, 2007, in Washington, DC.



John R. Hutchins

EXHIBIT

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US006352861B1

(12) **United States Patent**
Copeland et al.

(10) **Patent No.:** **US 6,352,861 B1**
(45) **Date of Patent:** **Mar. 5, 2002**

(54) **AUTOMATED BIOLOGICAL REACTION APPARATUS**

(75) Inventors: **Keith G. Copeland; Thomas M. Grogan; Charles Hassen; William Ross Humphreys; Charles E. Lemme; Phillip C. Miller; William L. Richards; Wayne A. Showalter**, all of Tucson, AZ (US)

(73) Assignee: **Ventana Medical Systems, Inc.**, Tucson, AZ (US)

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4,935,875	A	*	6/1990	Shah et al.	364/49
4,961,906	A	*	10/1990	Andersen et al.	422/102 7
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5,051,238	A		9/1991	Umetsu et al.		
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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(List continued on next page.)

(21) Appl. No.: **09/452,309**

(22) Filed: **Dec. 1, 1999**

Stark et al., An Automated Device of Immunocytochemistry, Journal of Immunological Methods, 1988, Elsevier, 107, pp. 89–92.*

Related U.S. Application Data

(63) Continuation of application No. 08/906,678, filed on Aug. 5, 1997, which is a continuation of application No. 08/479,415, filed on Jun. 6, 1995, now Pat. No. 5,654,200, which is a division of application No. 08/352,966, filed on Dec. 9, 1994, now Pat. No. 5,595,707, which is a continuation of application No. 07/924,052, filed on Aug. 31, 1992, now abandoned, which is a continuation-in-part of application No. 07/488,601, filed on Mar. 2, 1990, now abandoned.

(51) **Int. Cl.⁷** **G01N 1/00; G01N 35/04**

(52) **U.S. Cl.** **436/46; 436/43; 436/45; 436/47; 436/49; 436/54; 436/180; 422/63; 422/64; 422/65; 422/67; 422/100; 422/102; 427/2.11; 141/130; 141/145**

(58) **Field of Search** **422/63–65, 100, 422/67, 102; 436/43, 45, 46, 47, 49, 54, 180; 427/2.11; 141/130, 145**

Primary Examiner—Jill Warden
Assistant Examiner—Kathryn Bex
(74) *Attorney, Agent, or Firm*—McDonnell Boehnen Hulbert & Berghoff

(57) **ABSTRACT**

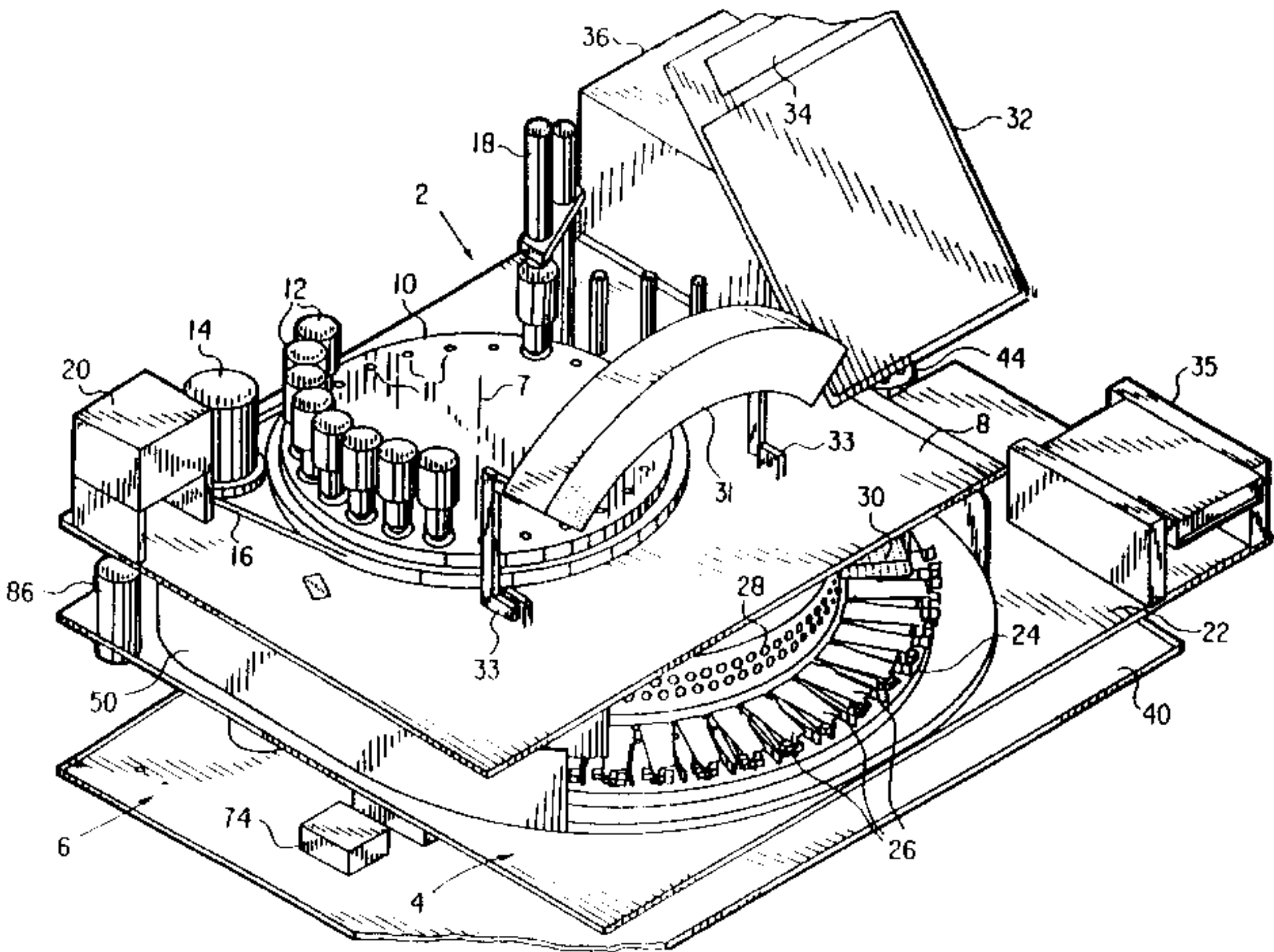
An automated immunostaining apparatus having a reagent application zone and a reagent supply zone. The apparatus has a carousel slide support supporting a plurality of slide supports thereon, and drive means engaging the carousel slide support for consecutively positioning each of a plurality of slide supports in the reagent application zone. The apparatus also has a carousel reagent support having a plurality of reagent container supports thereon, and drive means engaging the carousel for rotating the carousel and positioning a preselected reagent container support in the reagent supply zone. The apparatus also has a reagent delivery actuator means positioned for engaging a reagent container positioned on a container support in the reagent delivery zone and initiating reagent delivery from the reagent container to a slide supported on a slide support in the reagent receiving zone.

(56) **References Cited**

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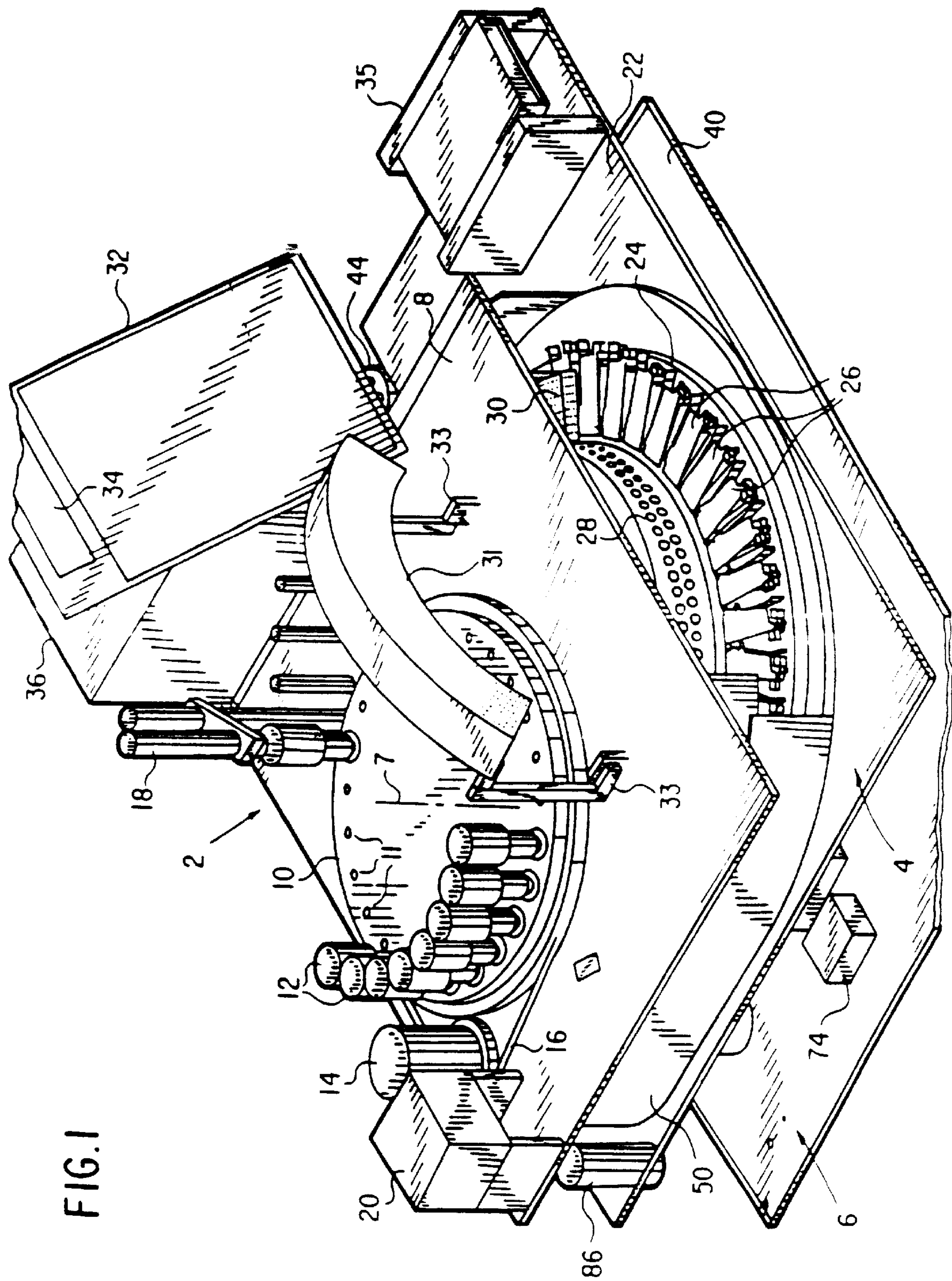
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25 Claims, 37 Drawing Sheets



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Page 2

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5,947,167	A *	9/1999	Bogen et al. 141/1
6,193,933	B1 *	2/2001	Sasaki et al. 422/64
* cited by examiner			



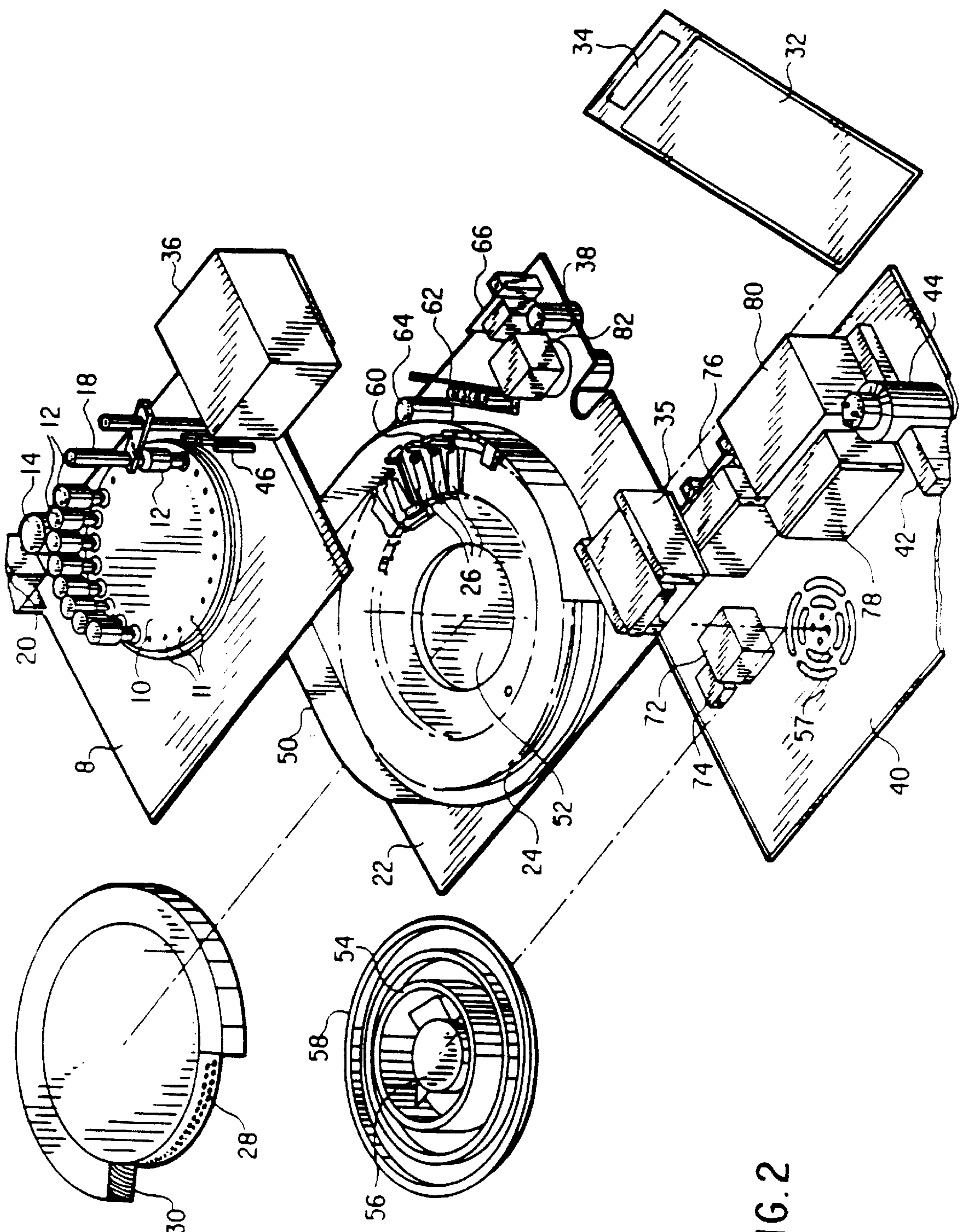


FIG. 2

FIG.3

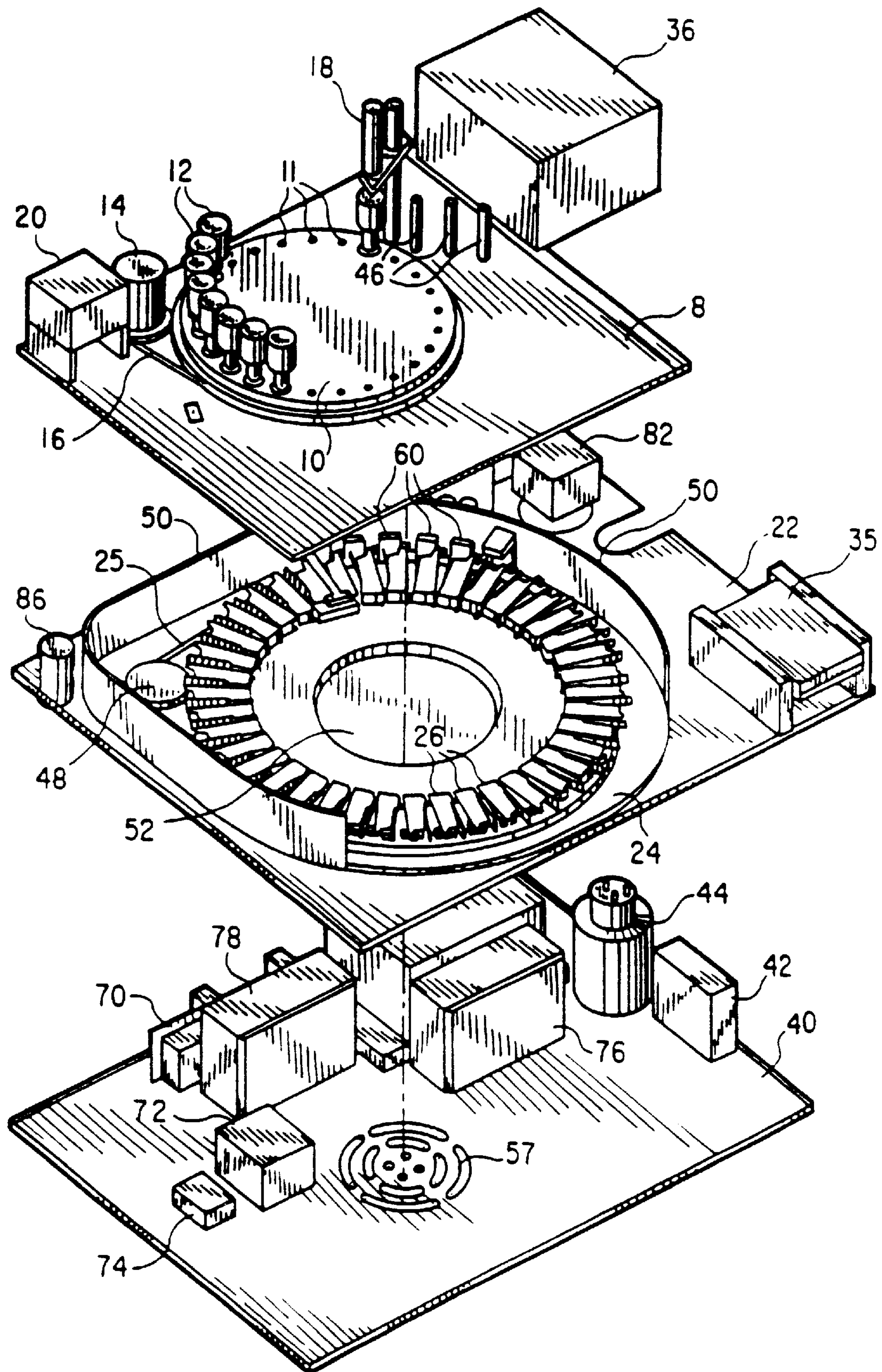
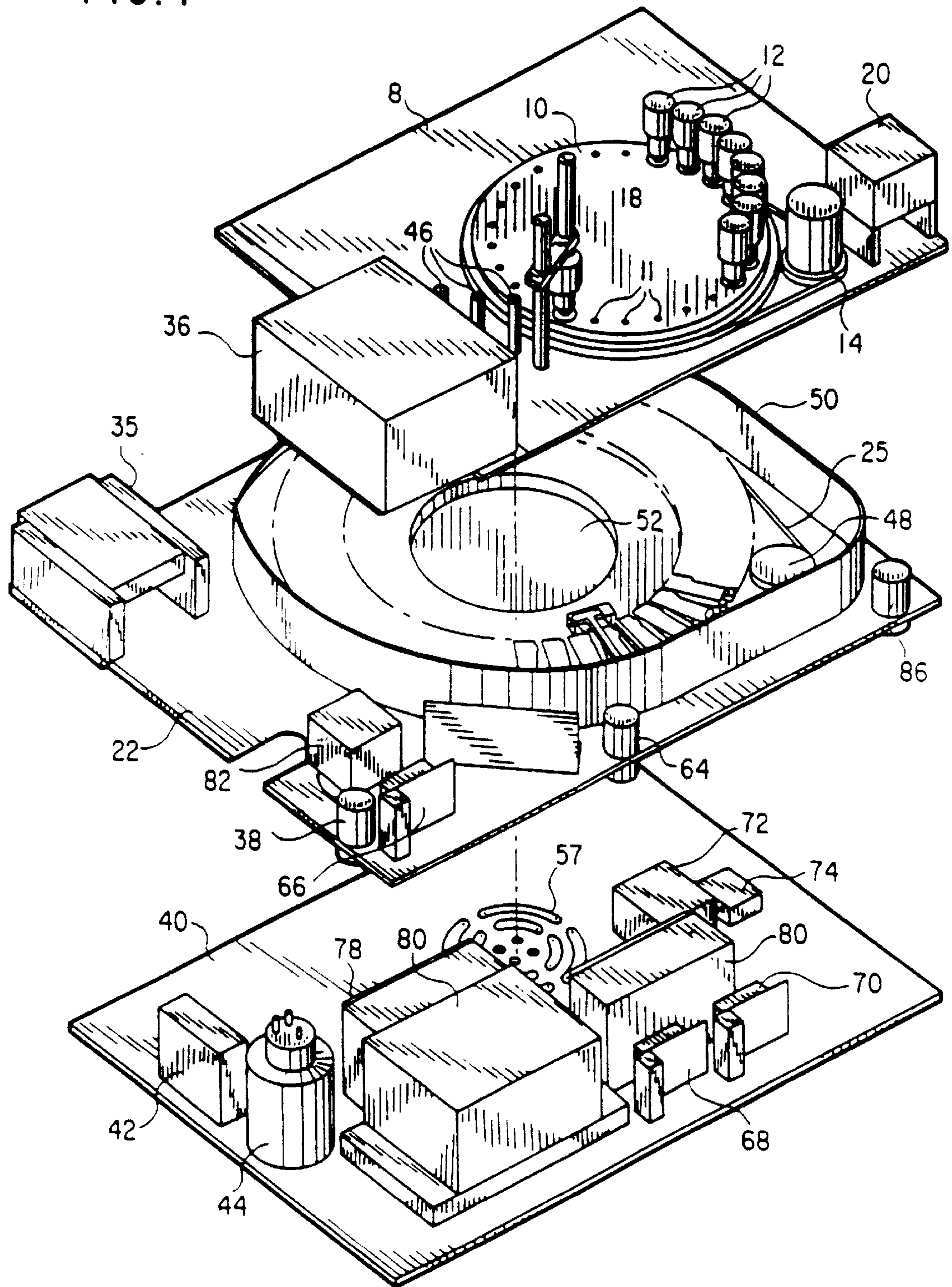


FIG. 4



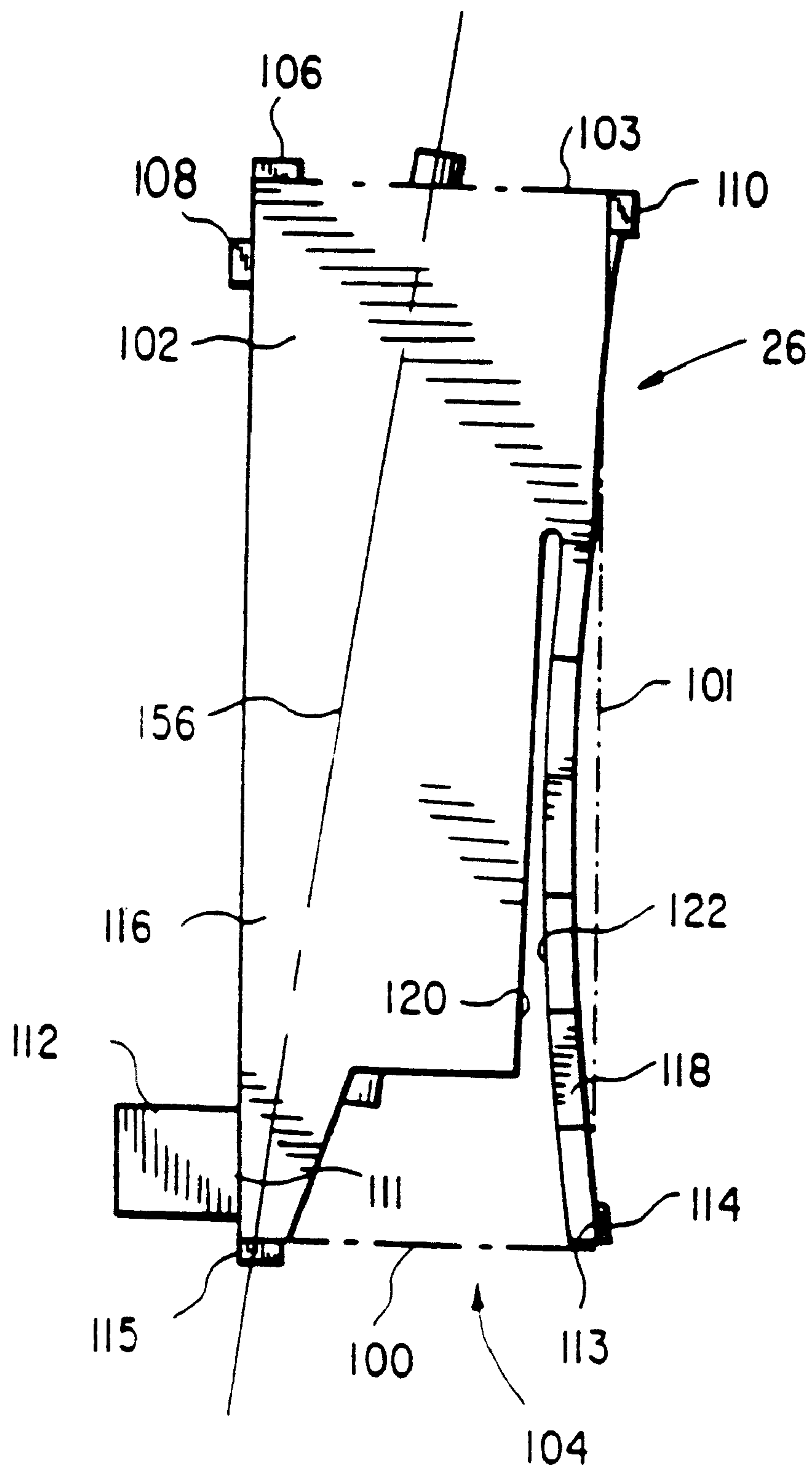


FIG. 5

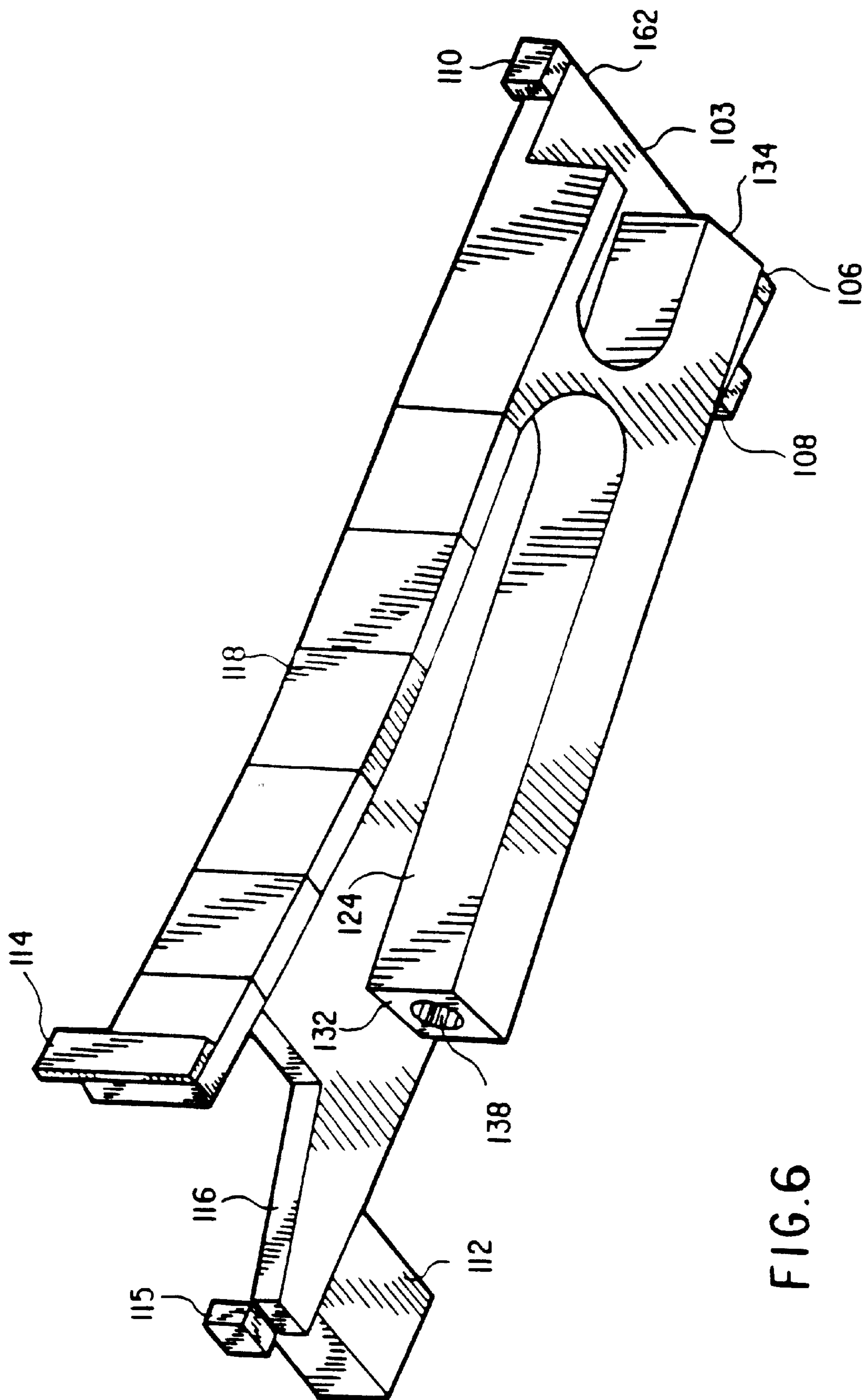


FIG. 6

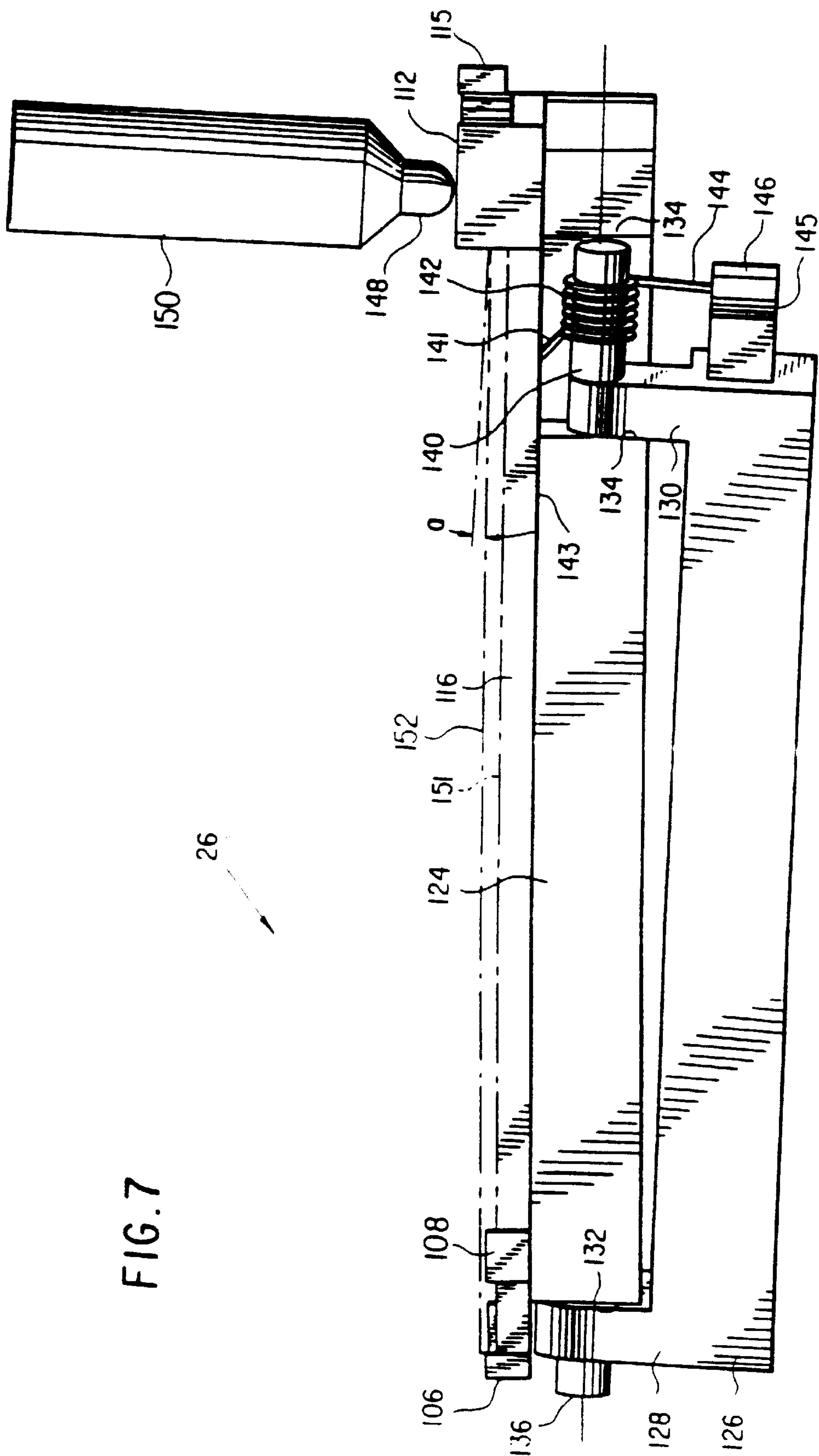
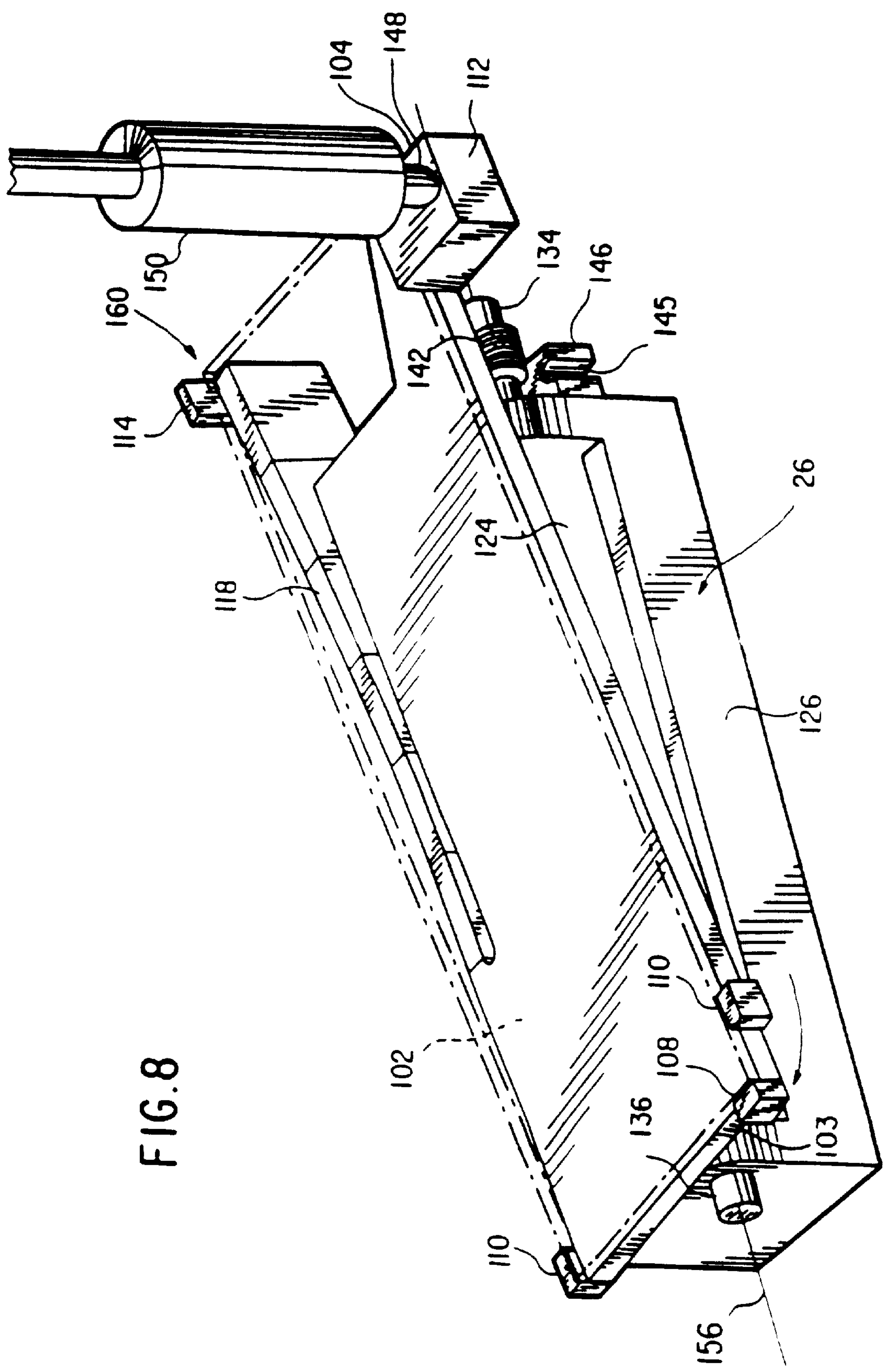


FIG. 8



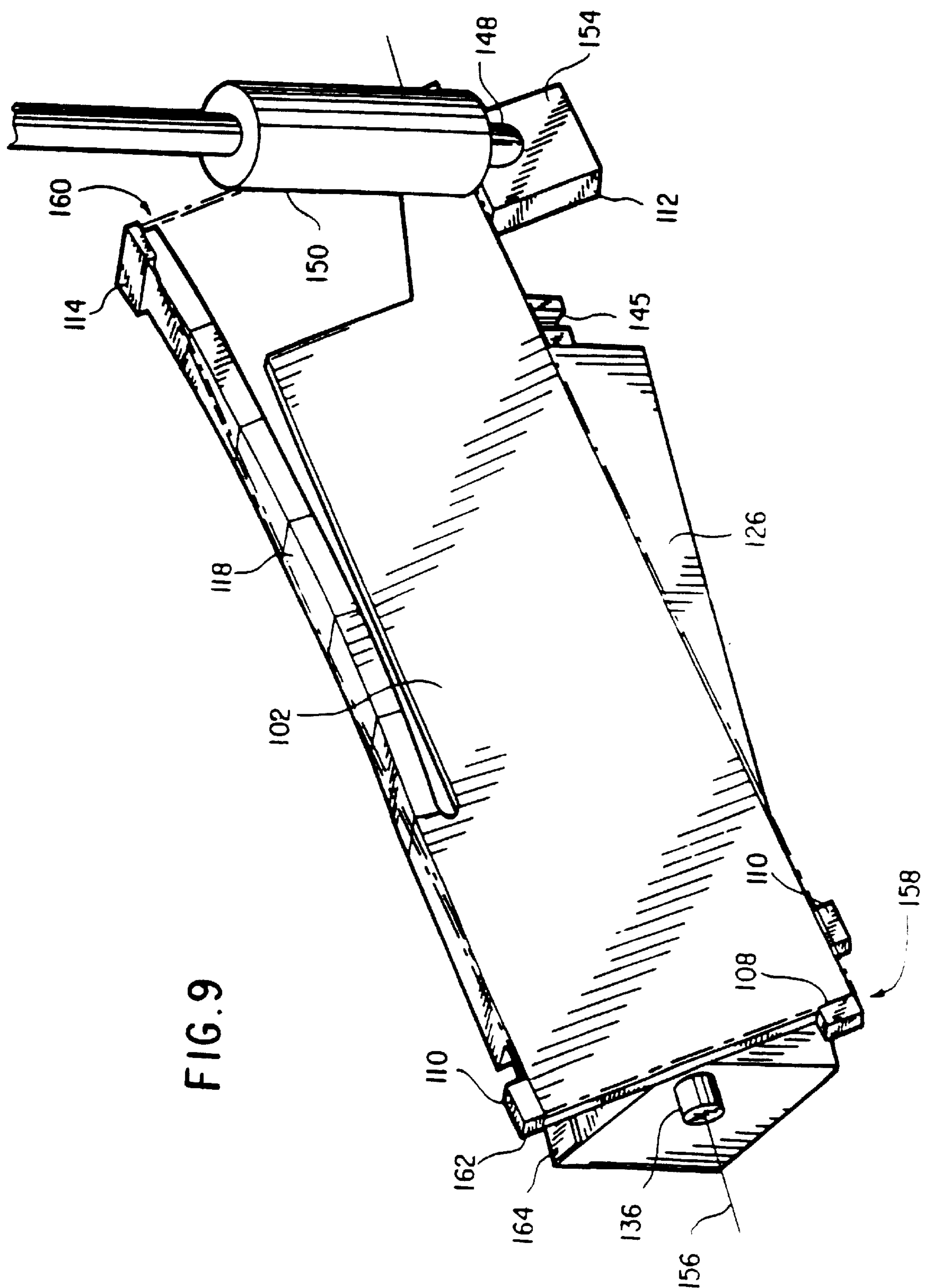
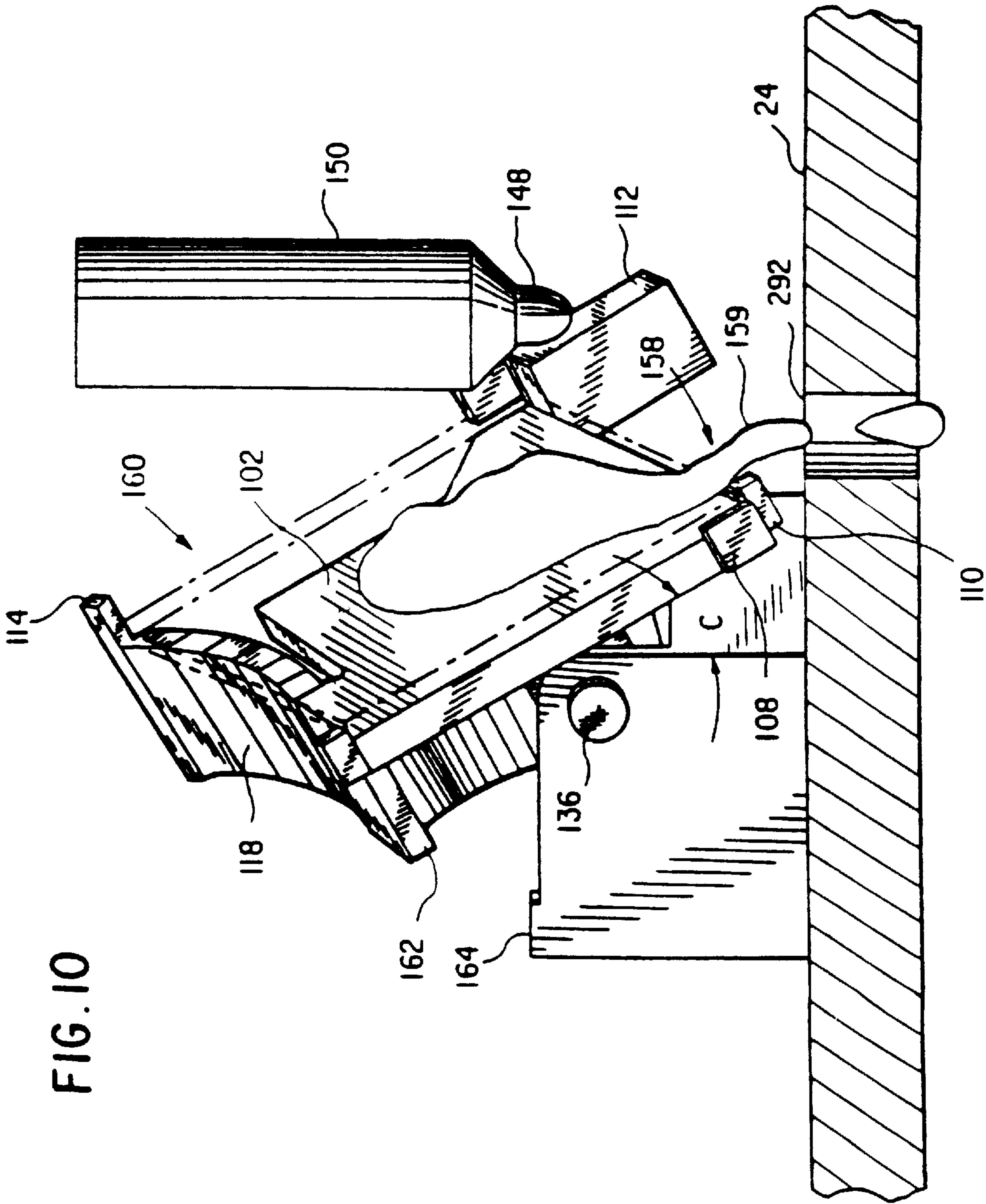
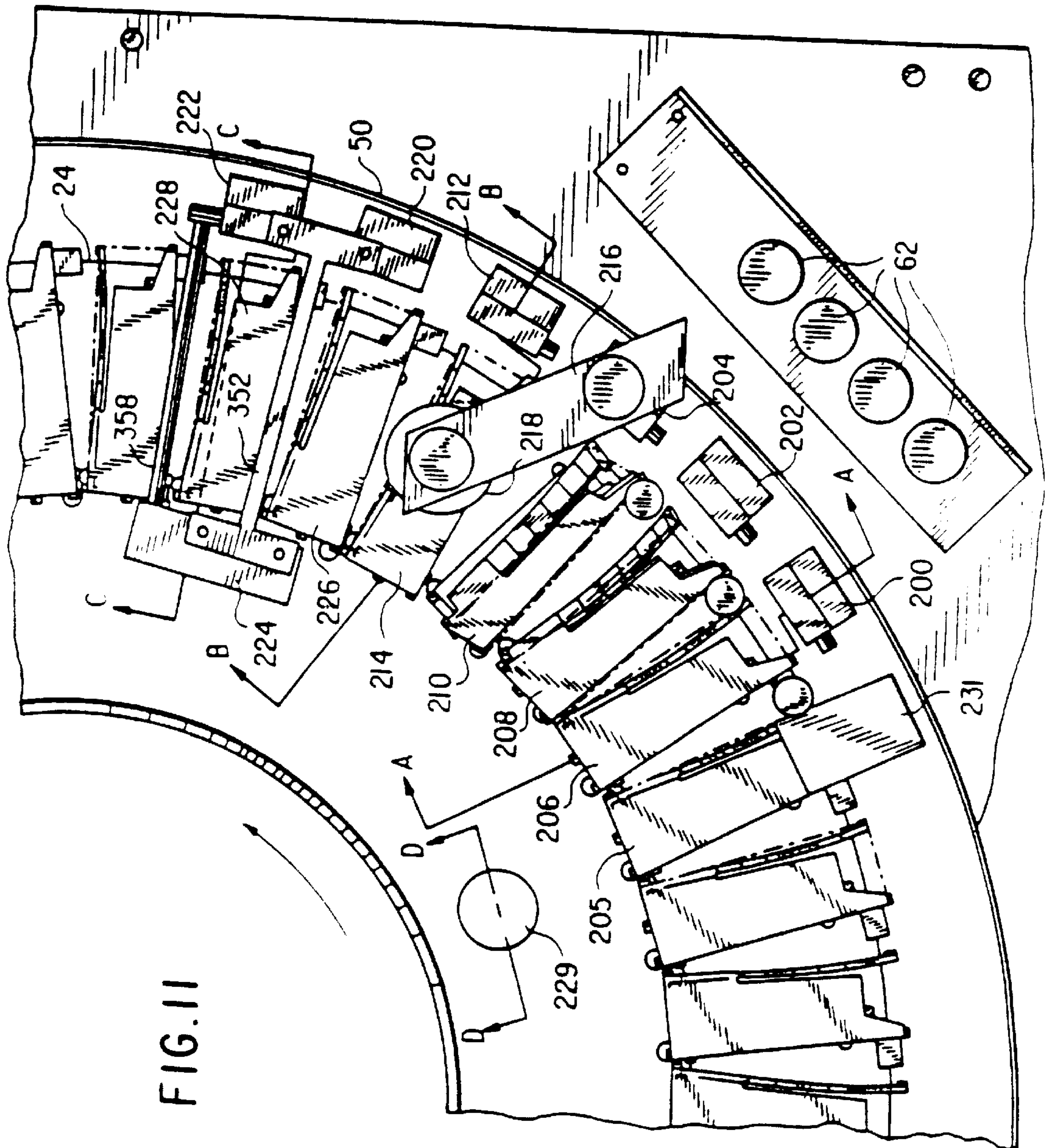


FIG. 9





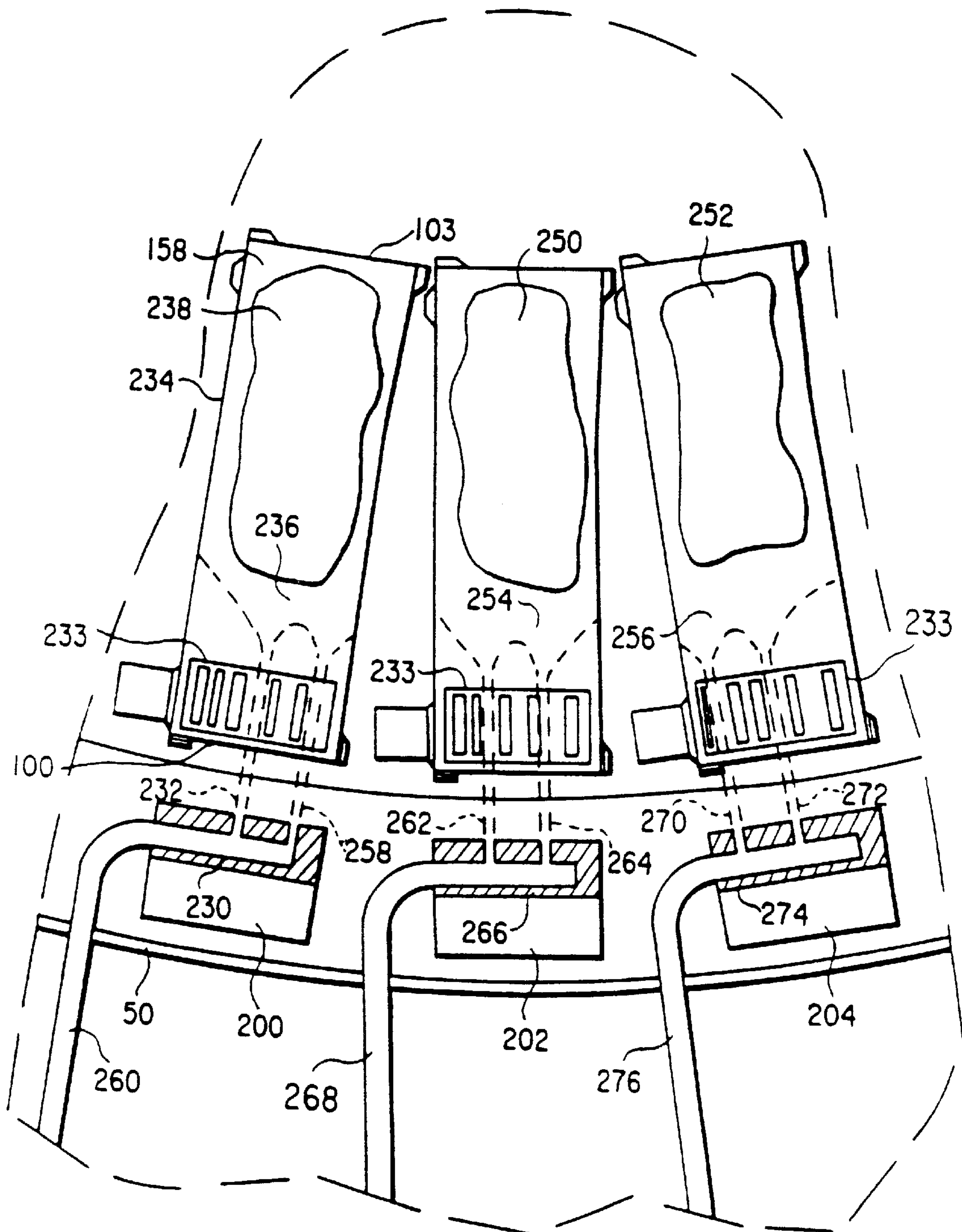
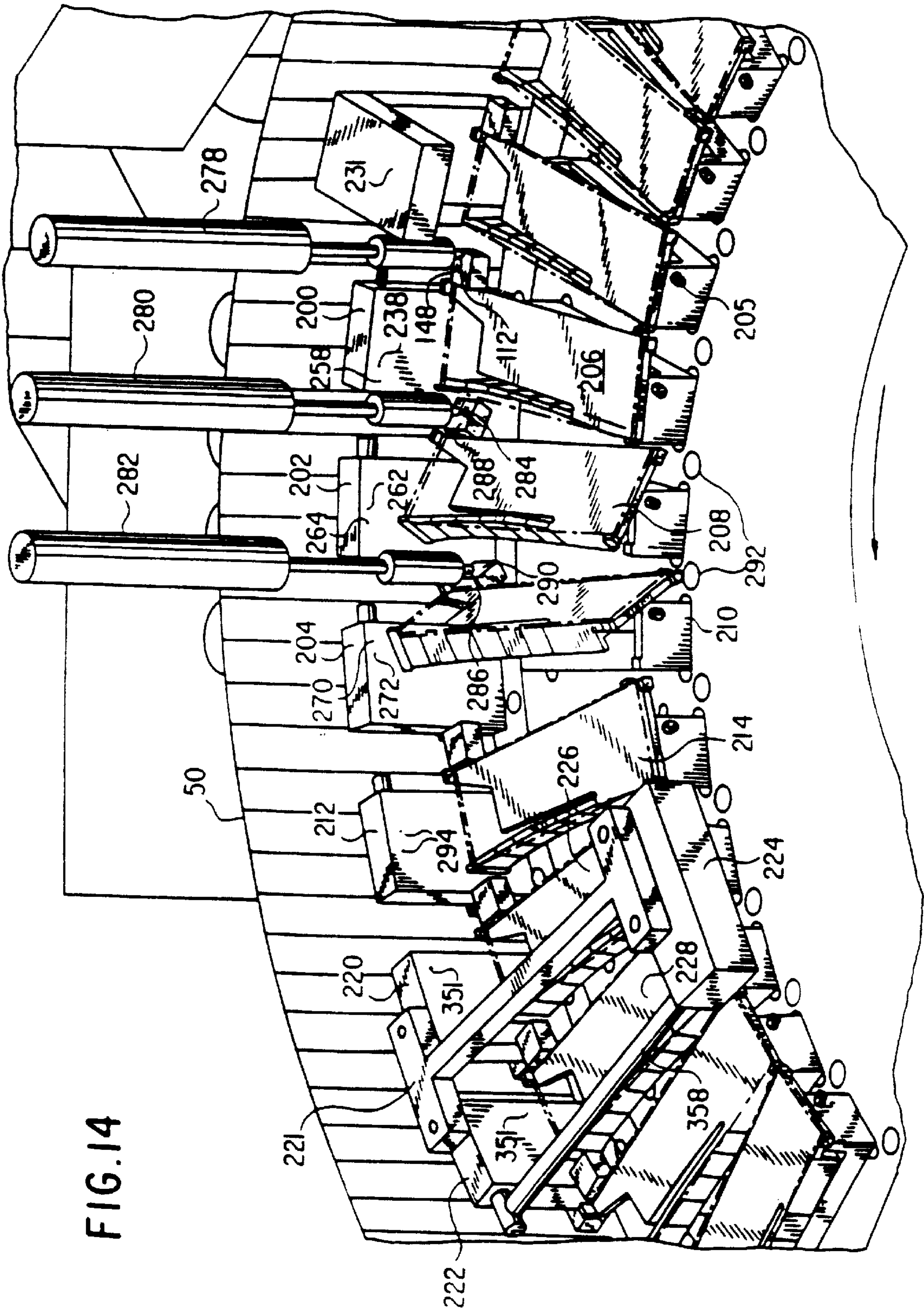


FIG. 13



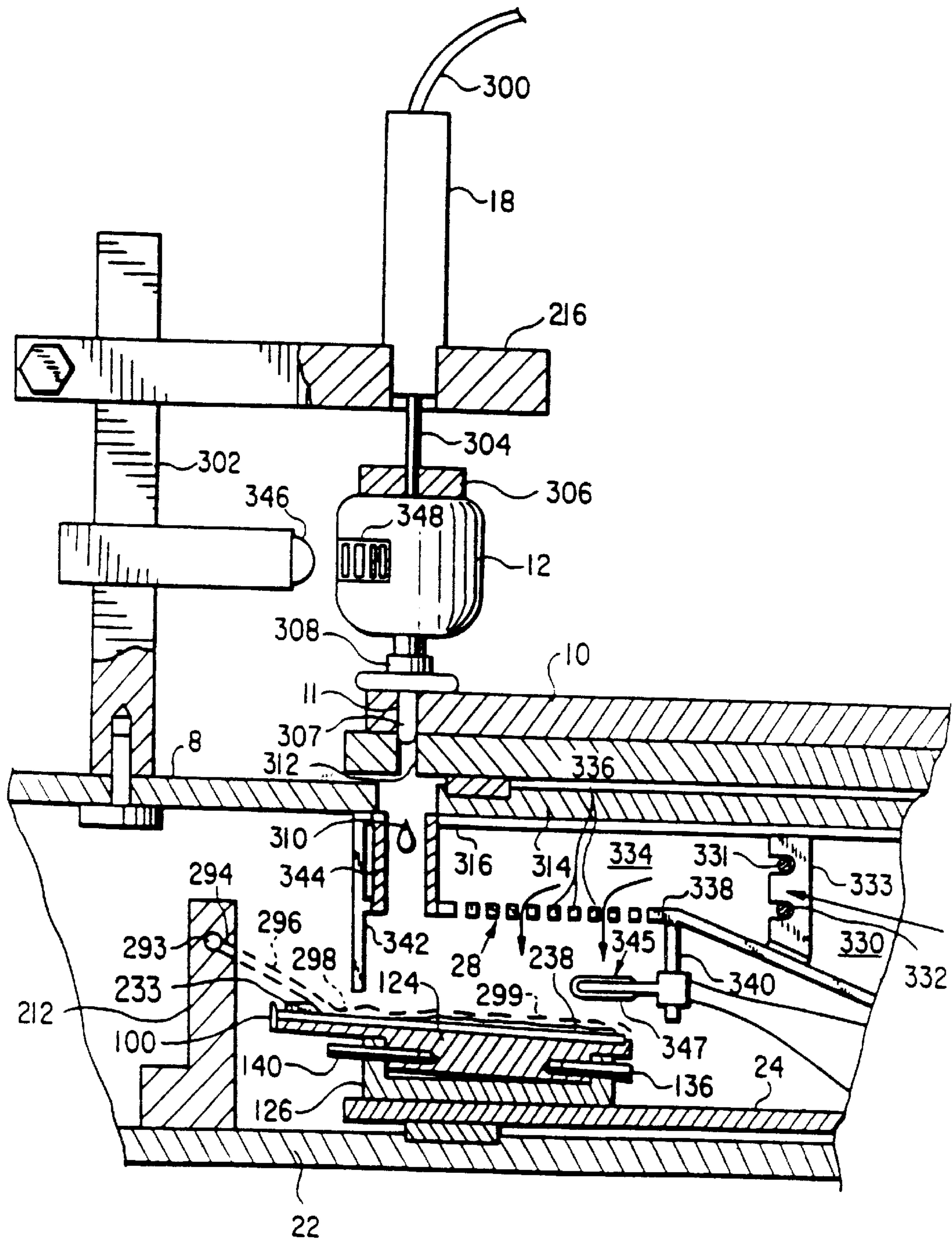


FIG. 15

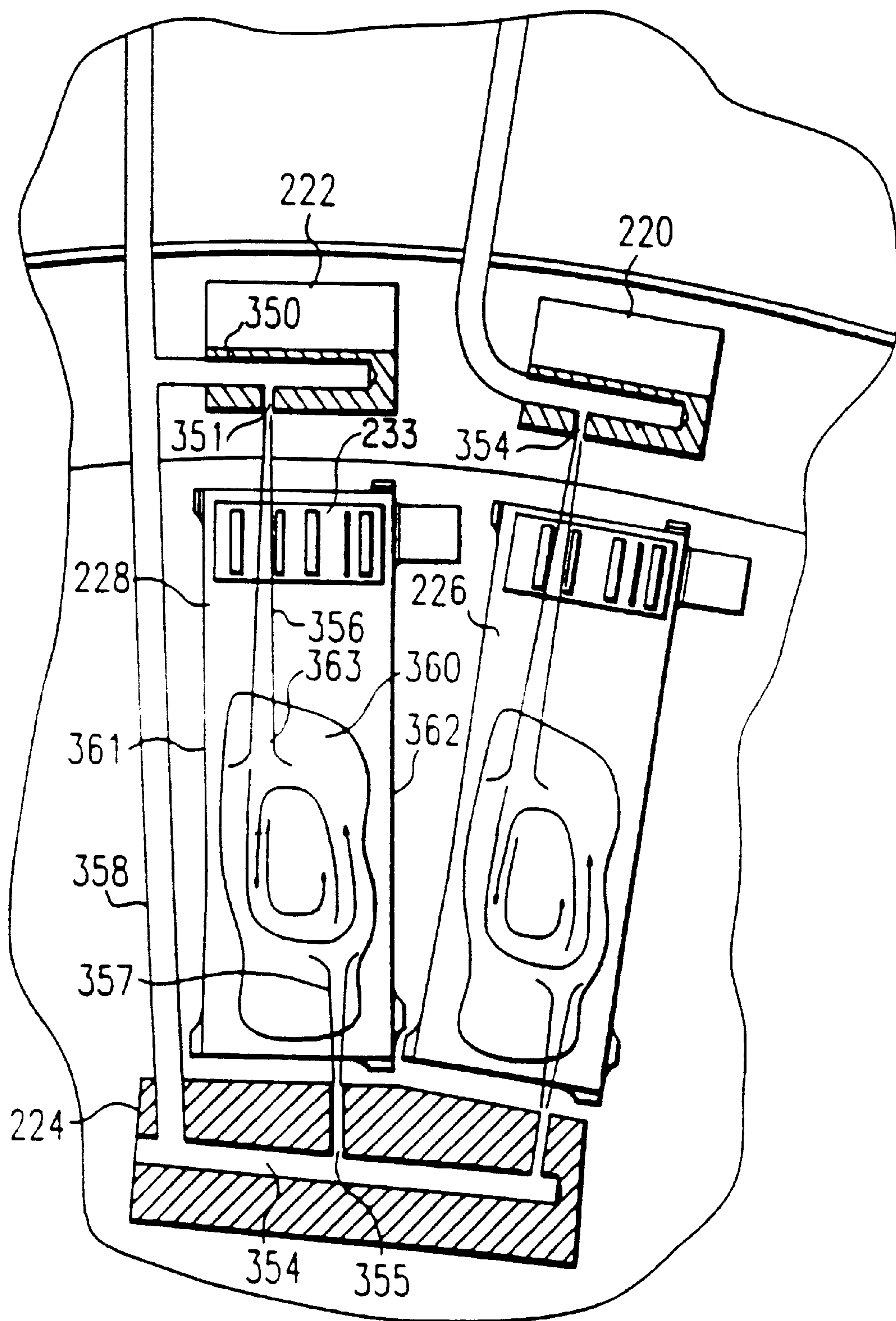


FIG. 17

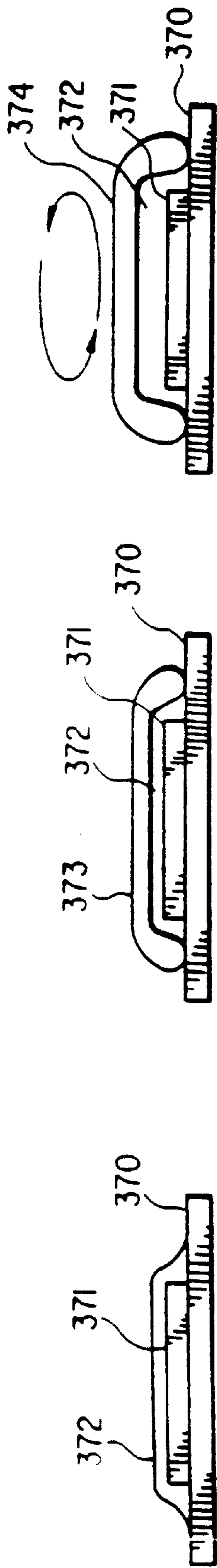


FIG. 18A

FIG. 18B

FIG. 18C

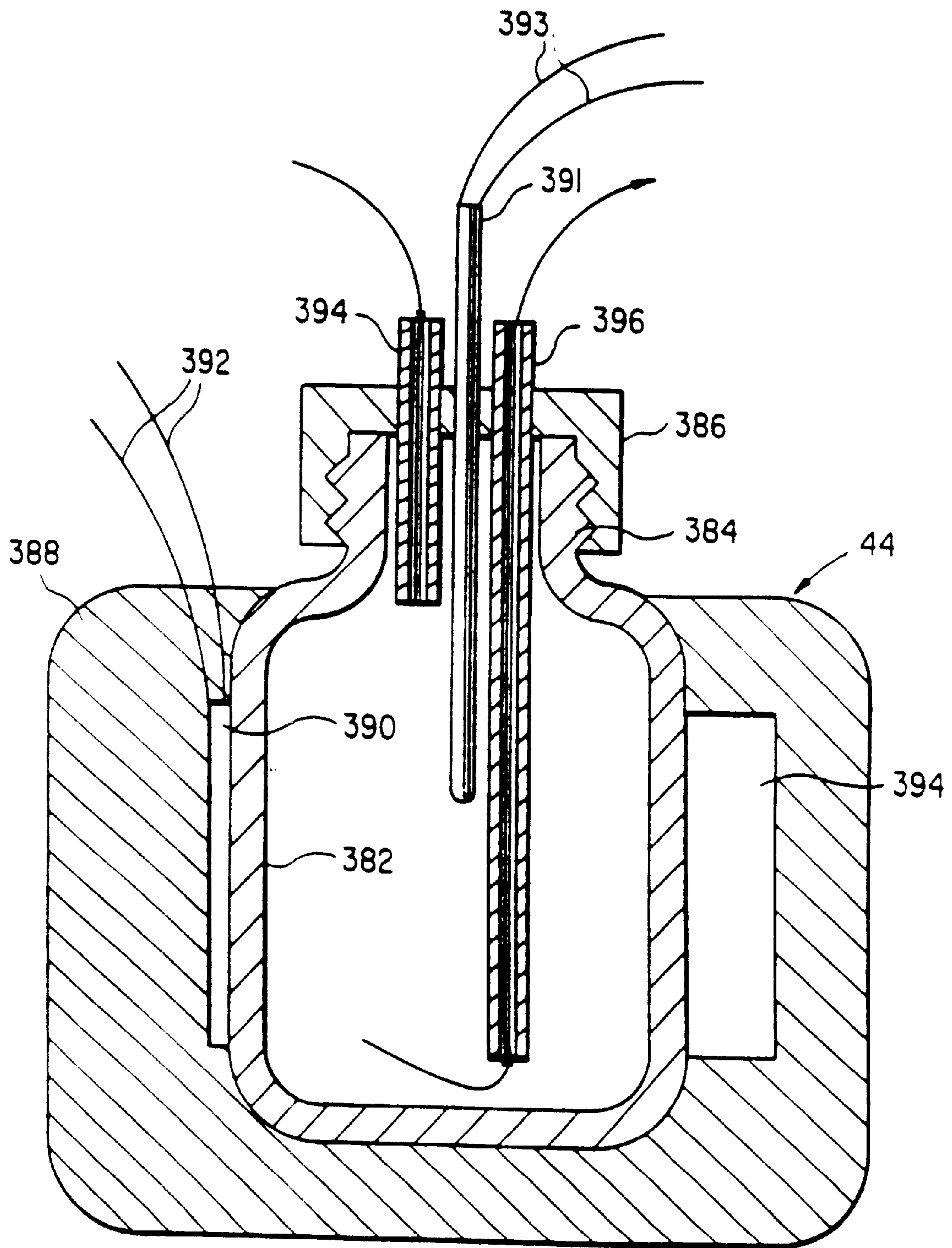


FIG. 19A

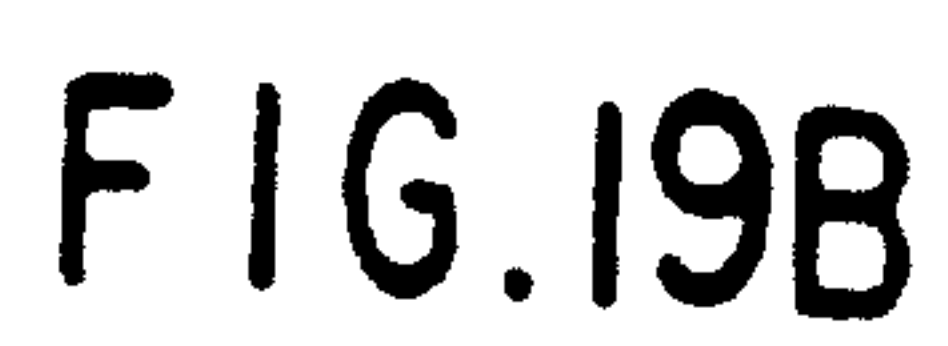


FIG. 19B

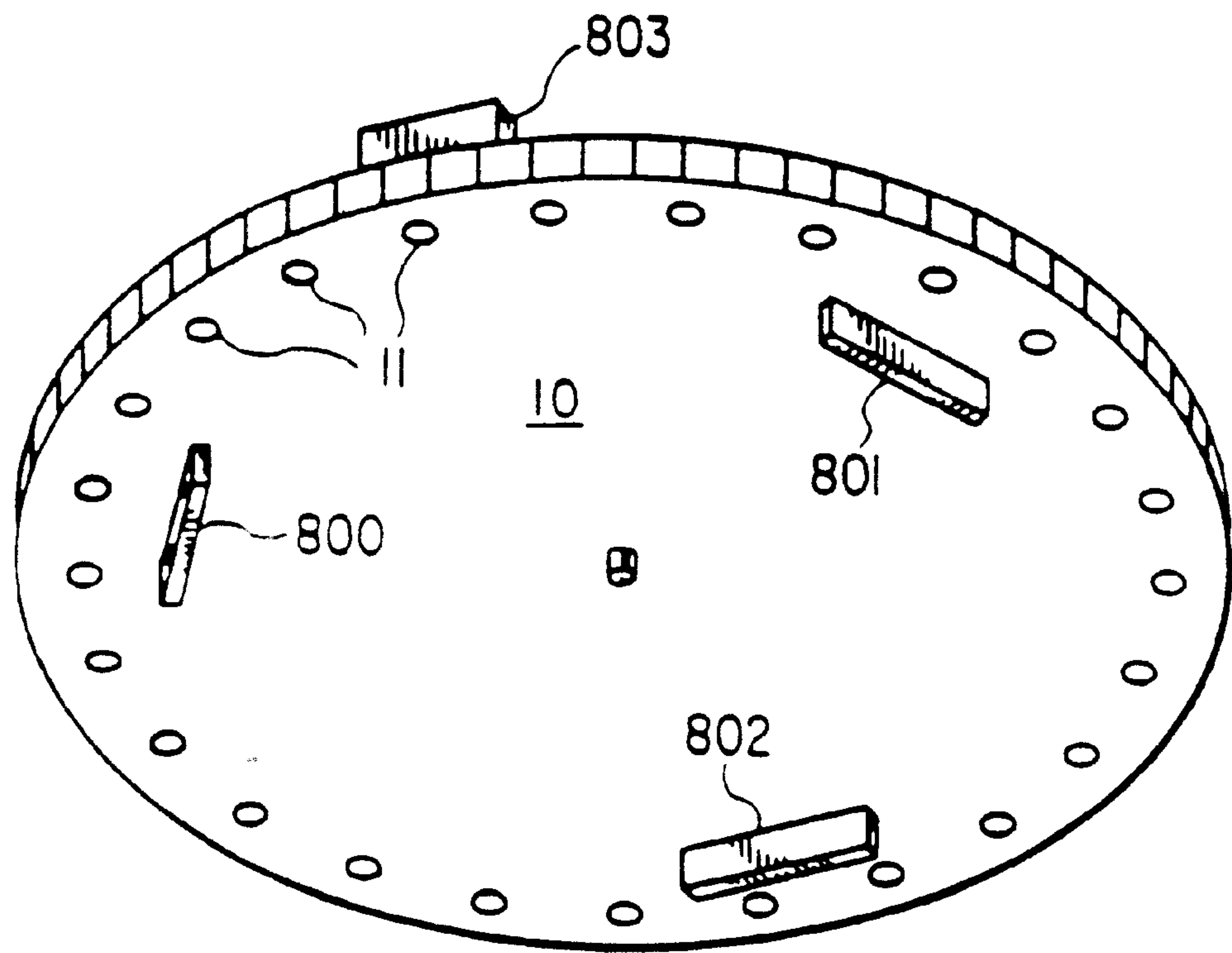


FIG. 20A

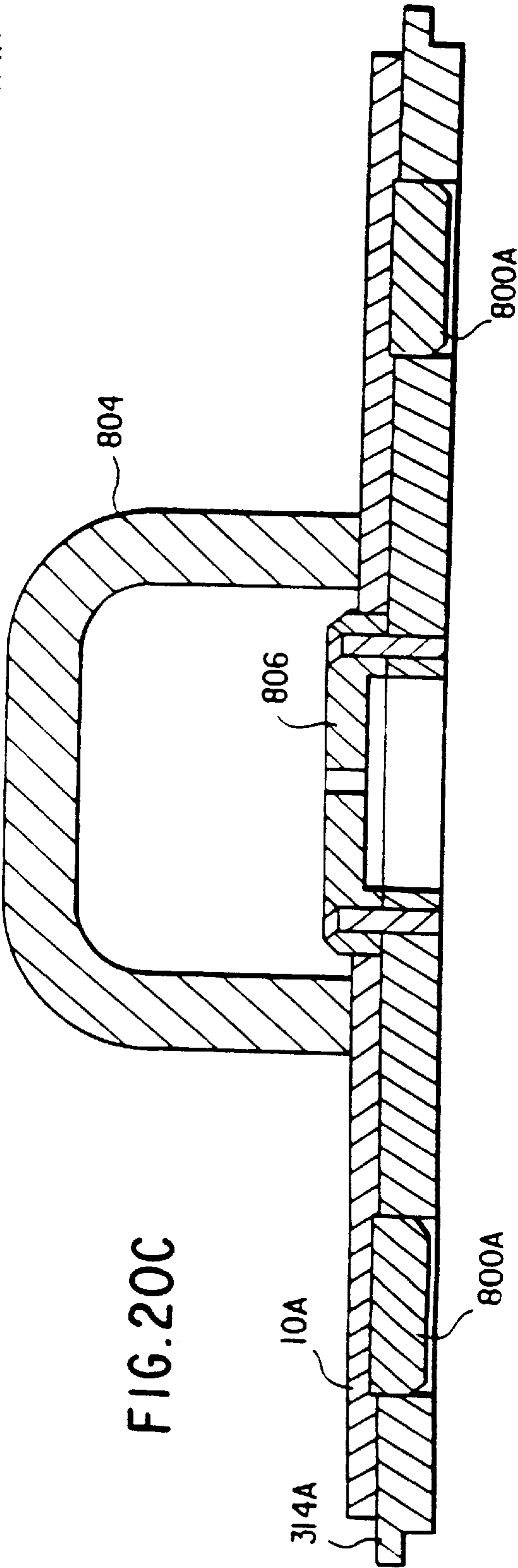
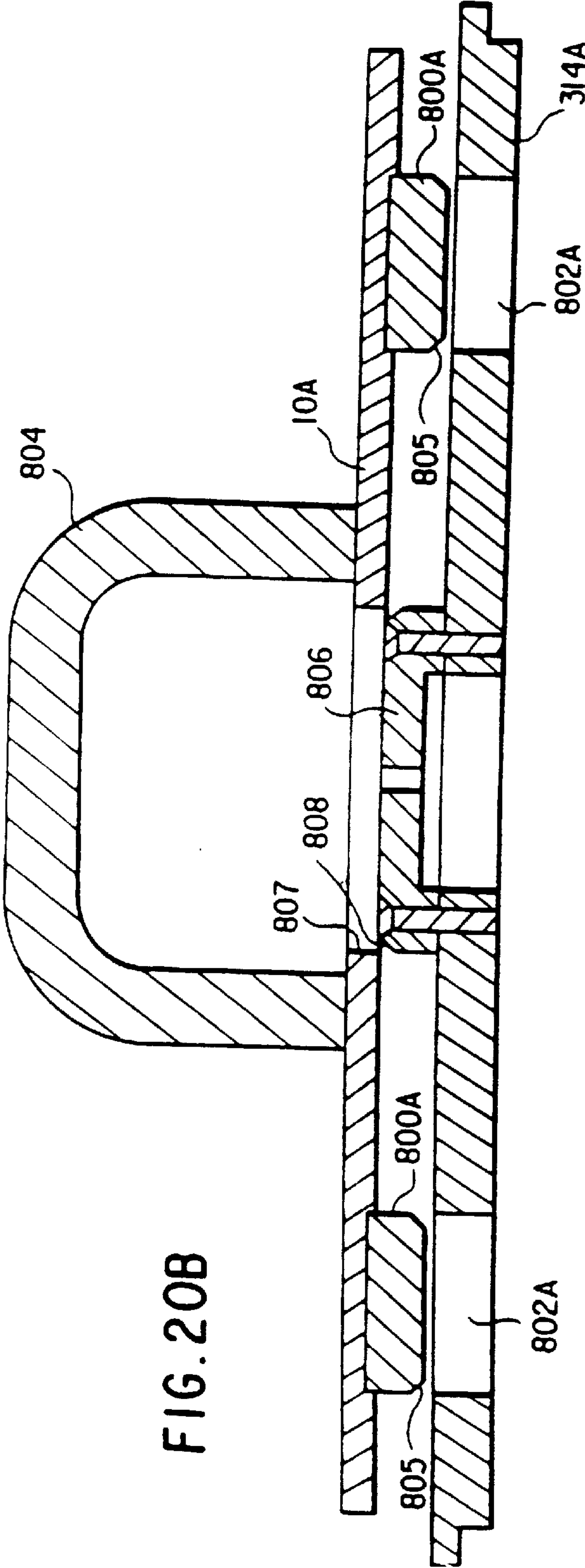
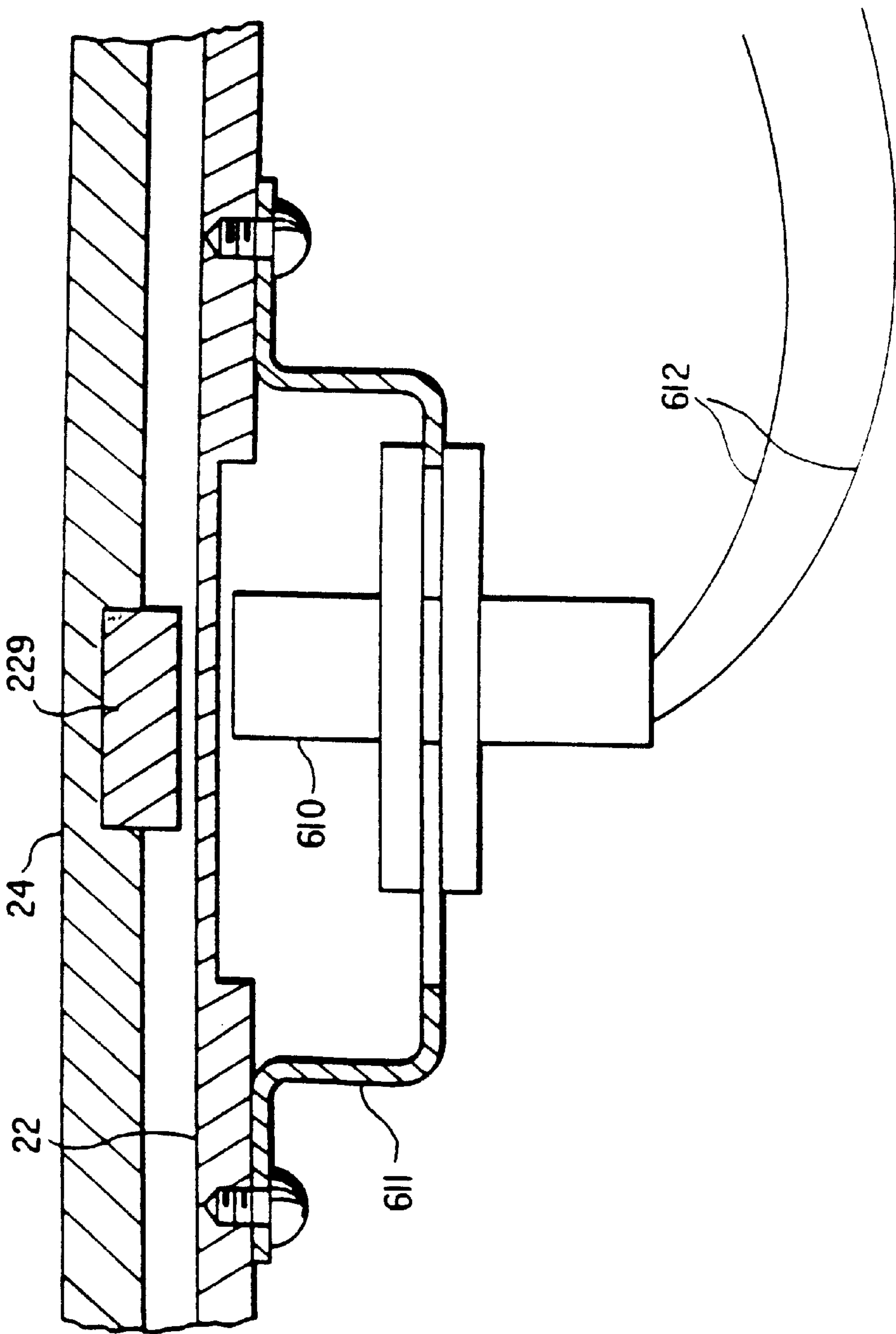
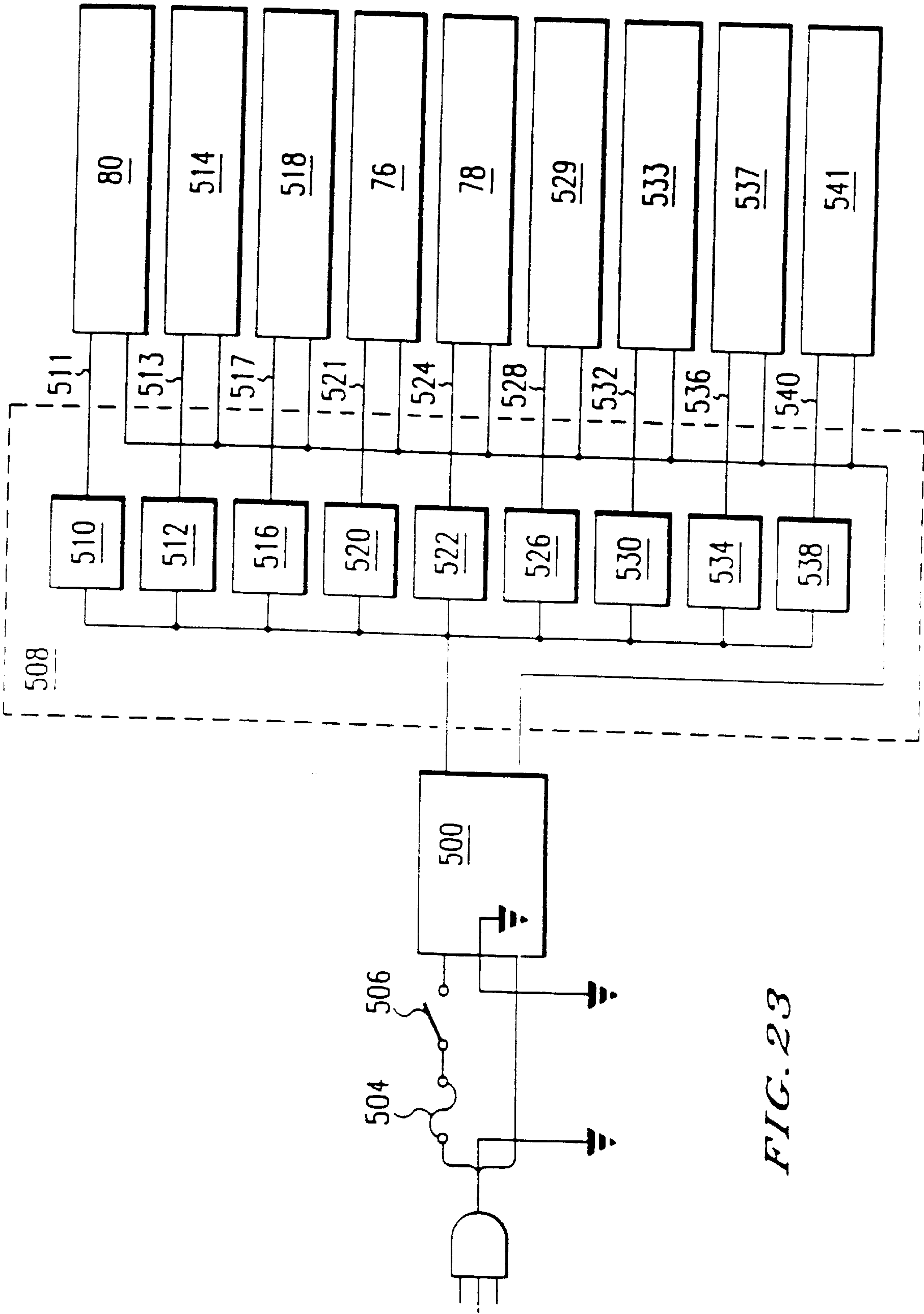


FIG. 21







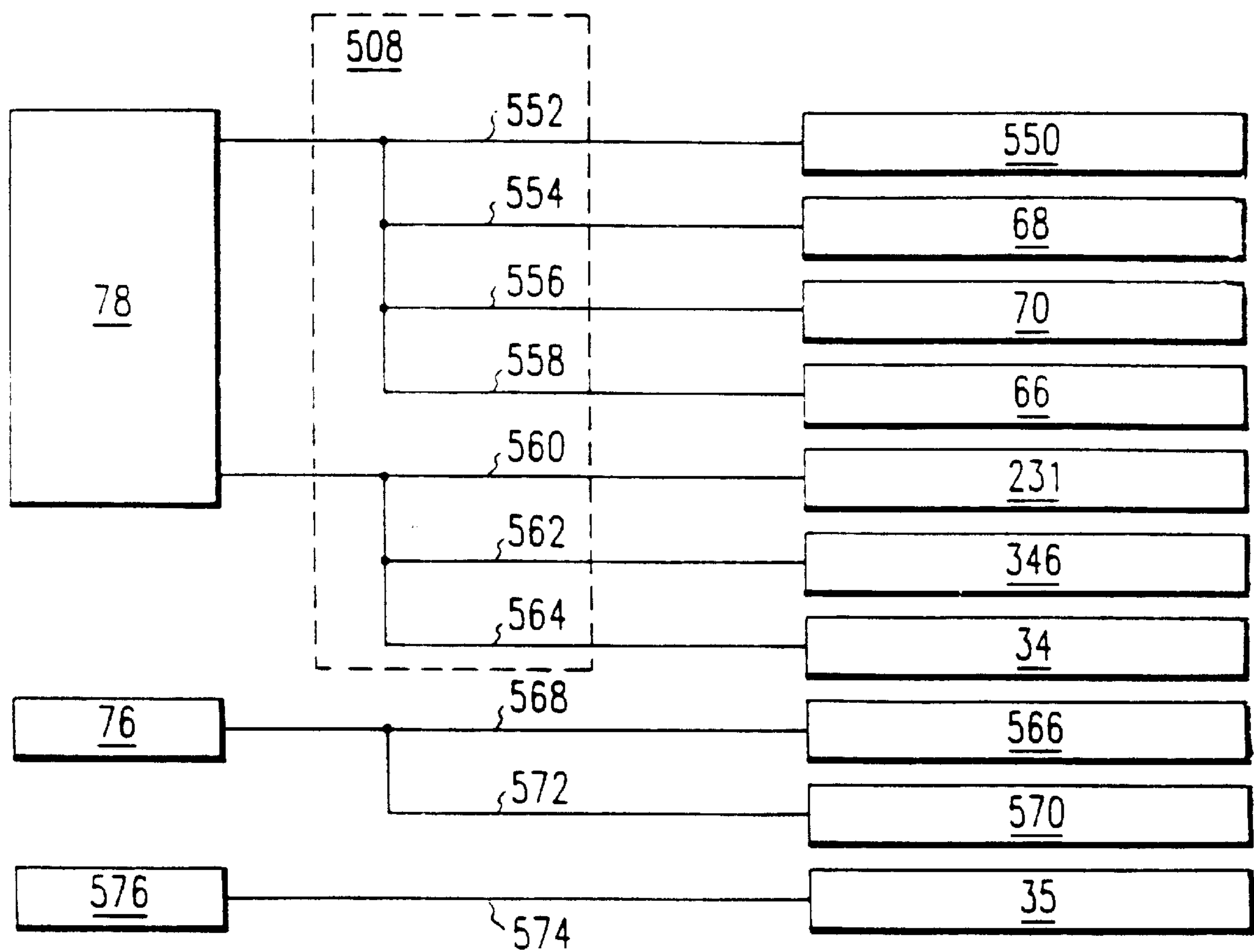
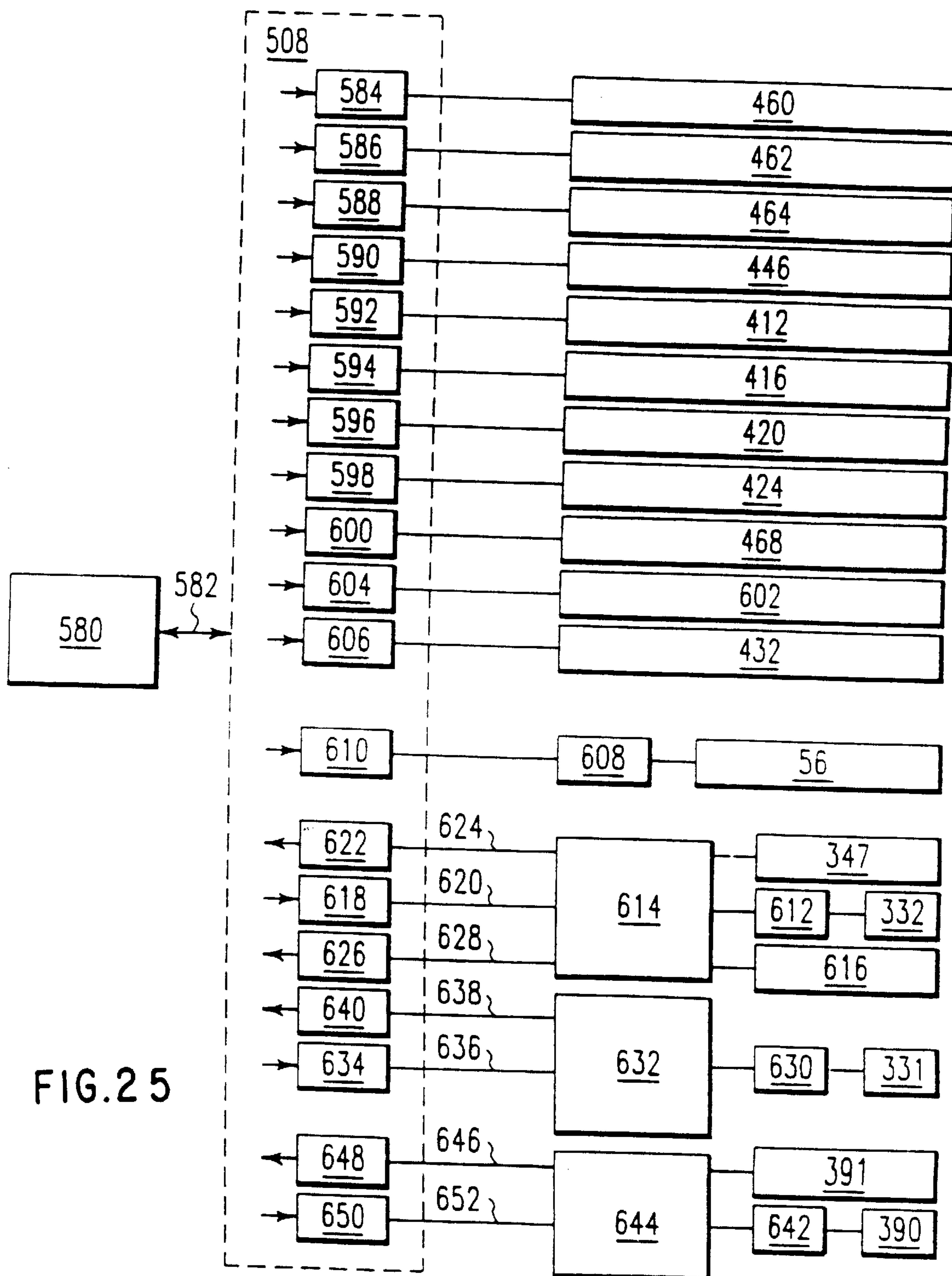


FIG. 24



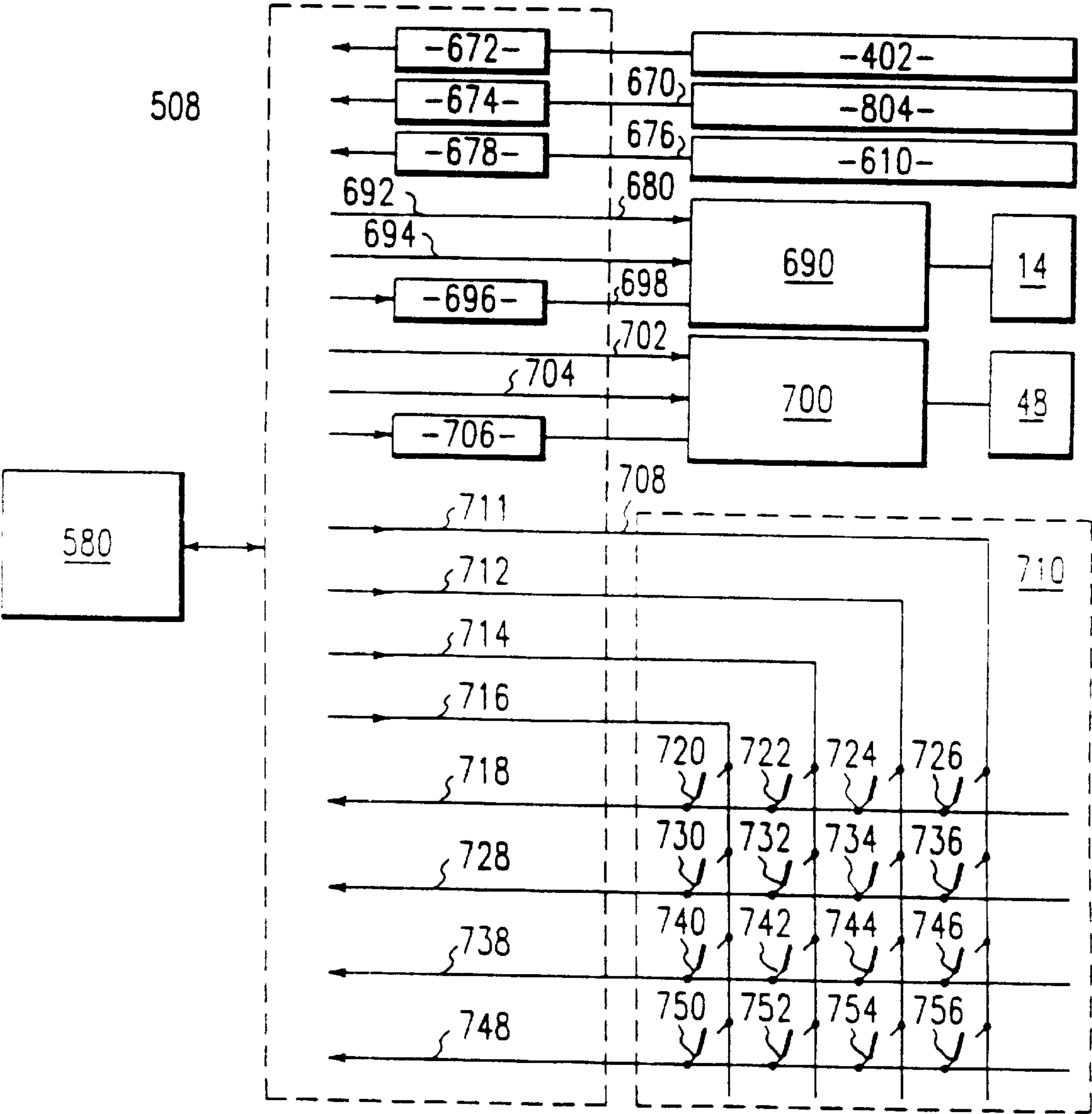


FIG. 26

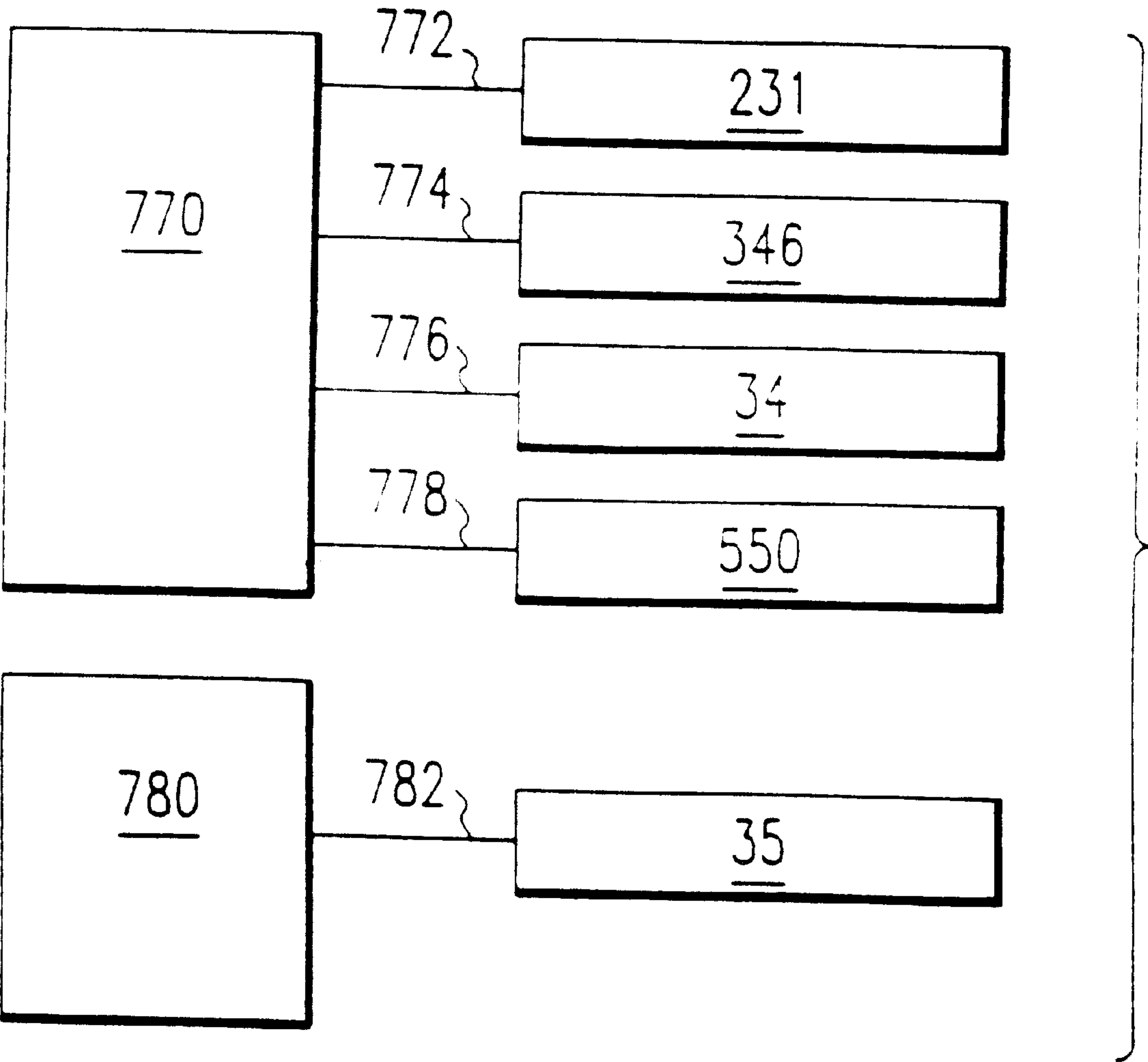


FIG. 27

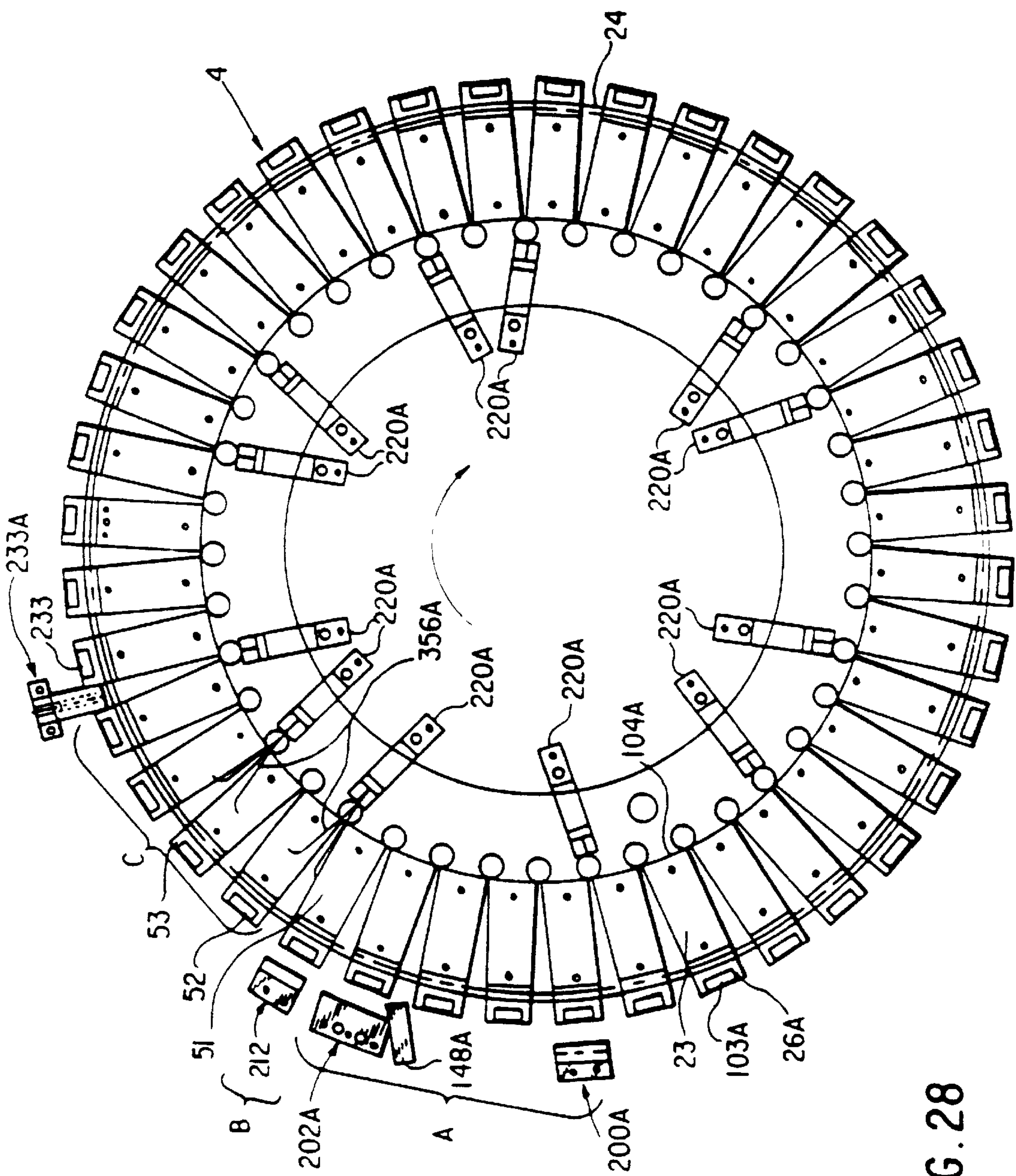


FIG. 28

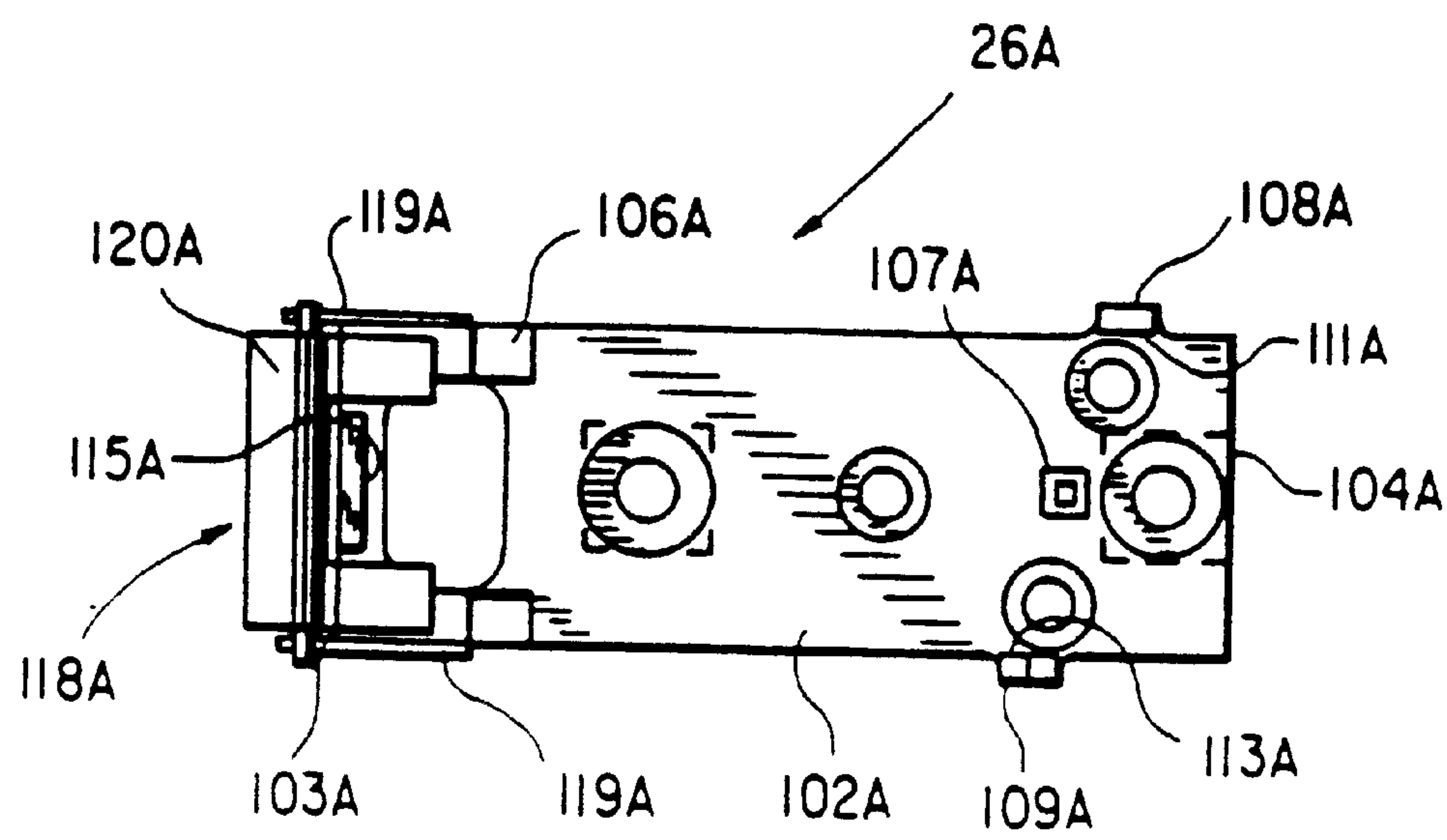


FIG. 29A

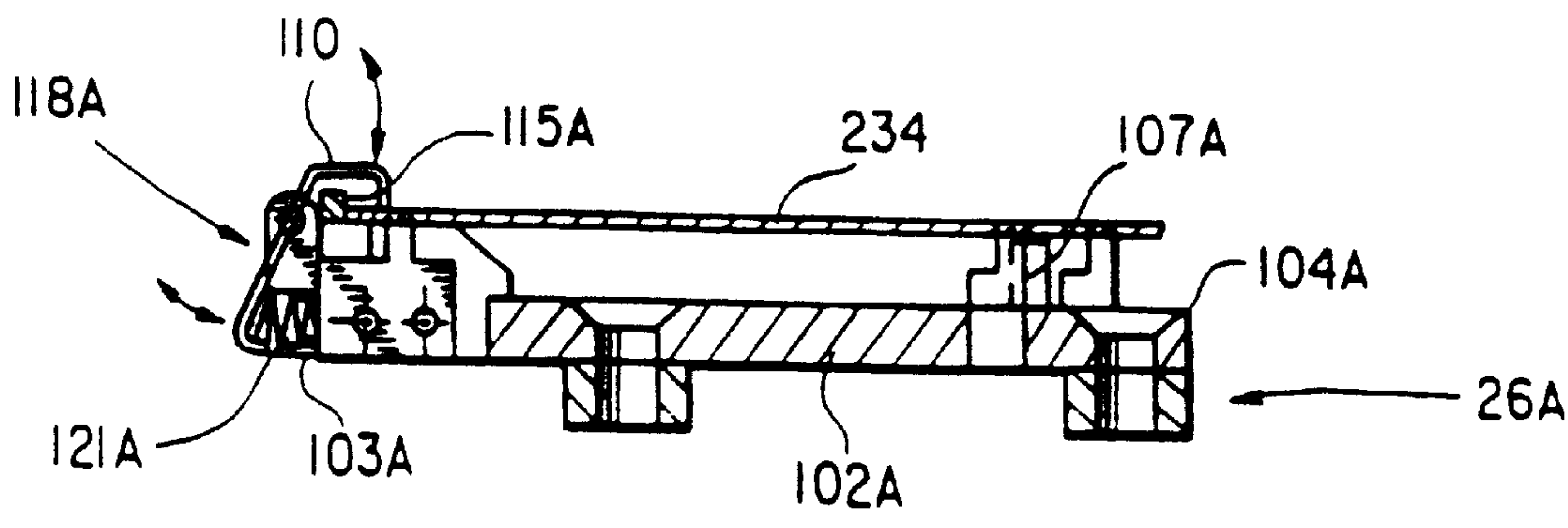
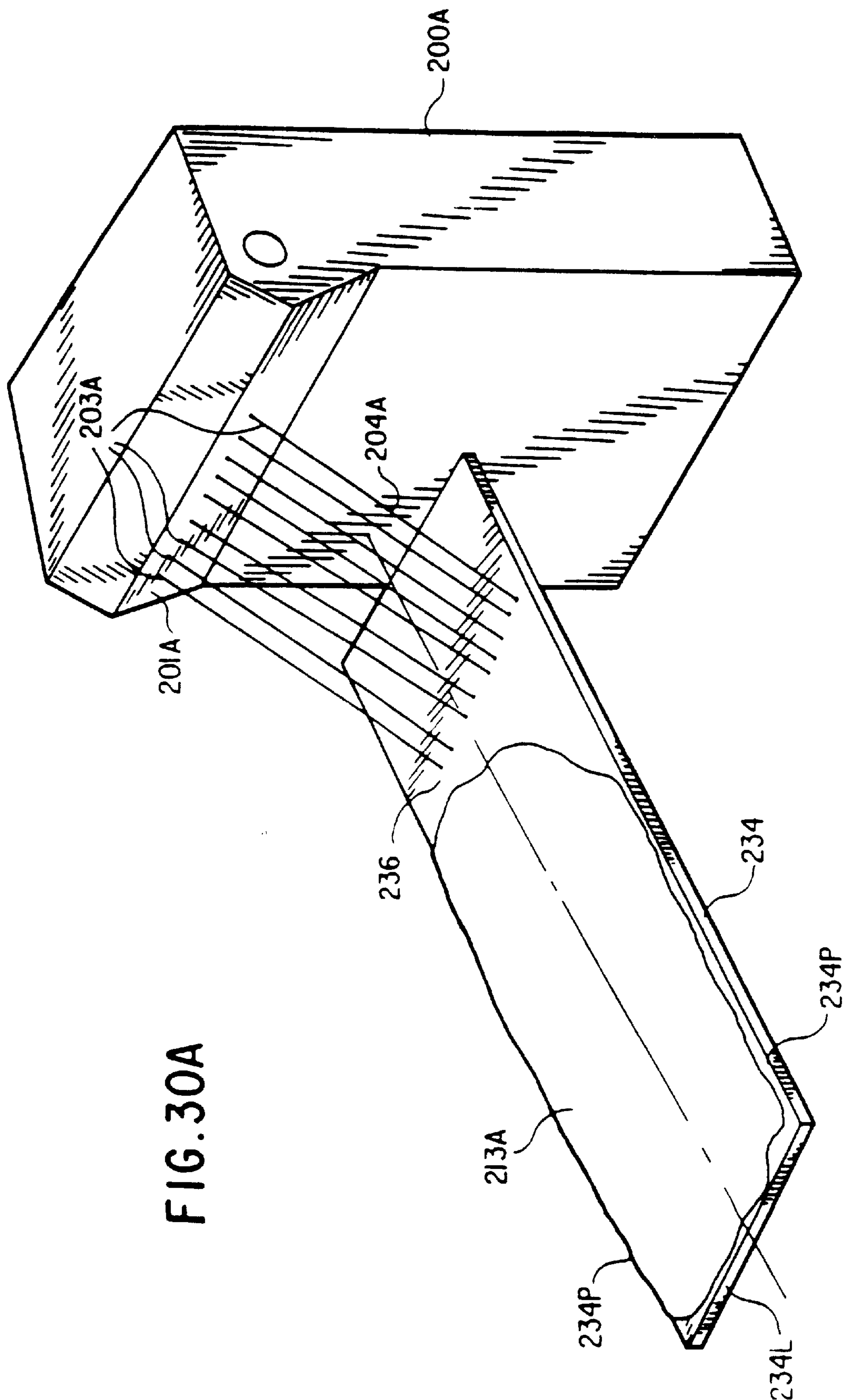


FIG. 29B



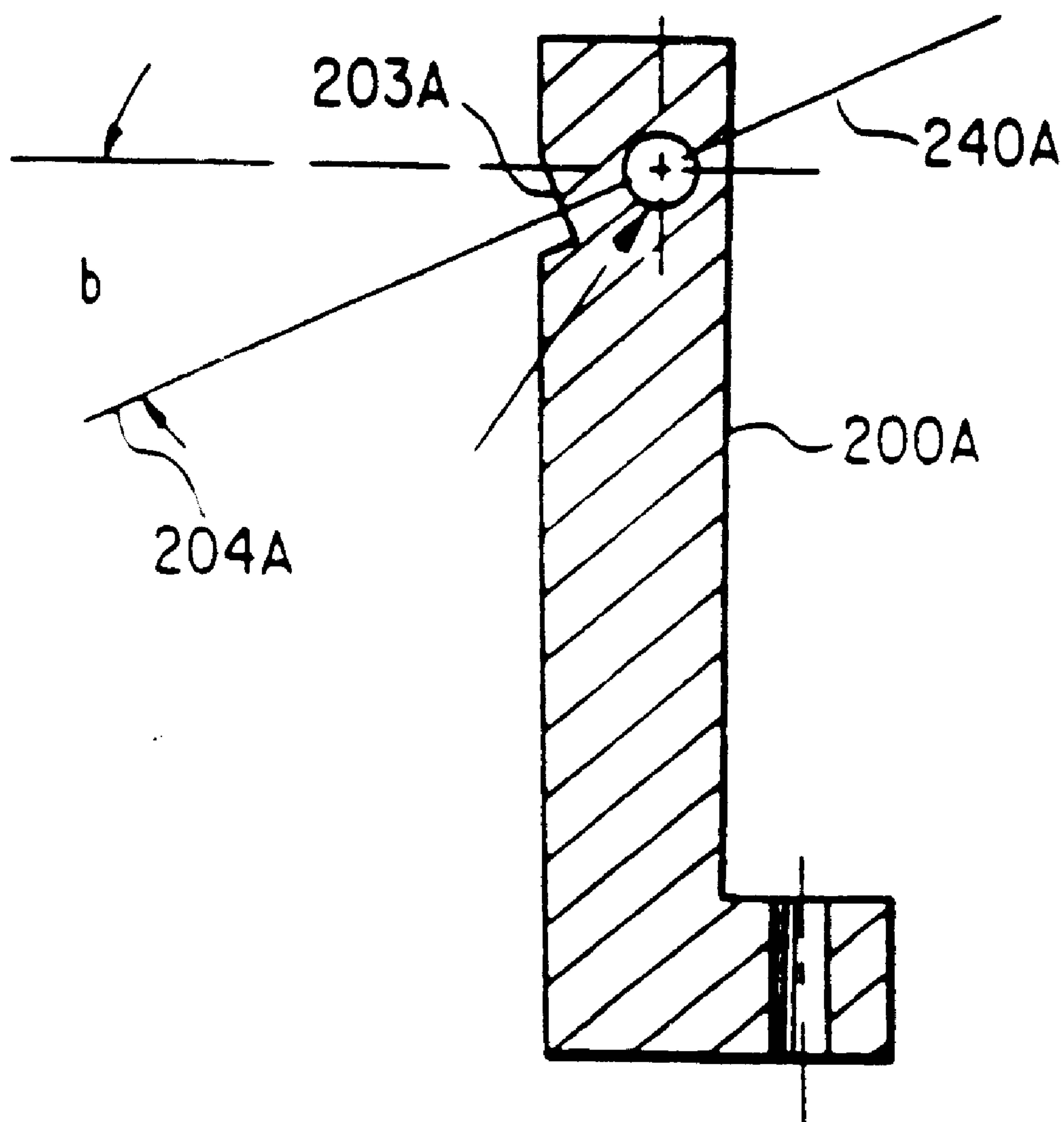
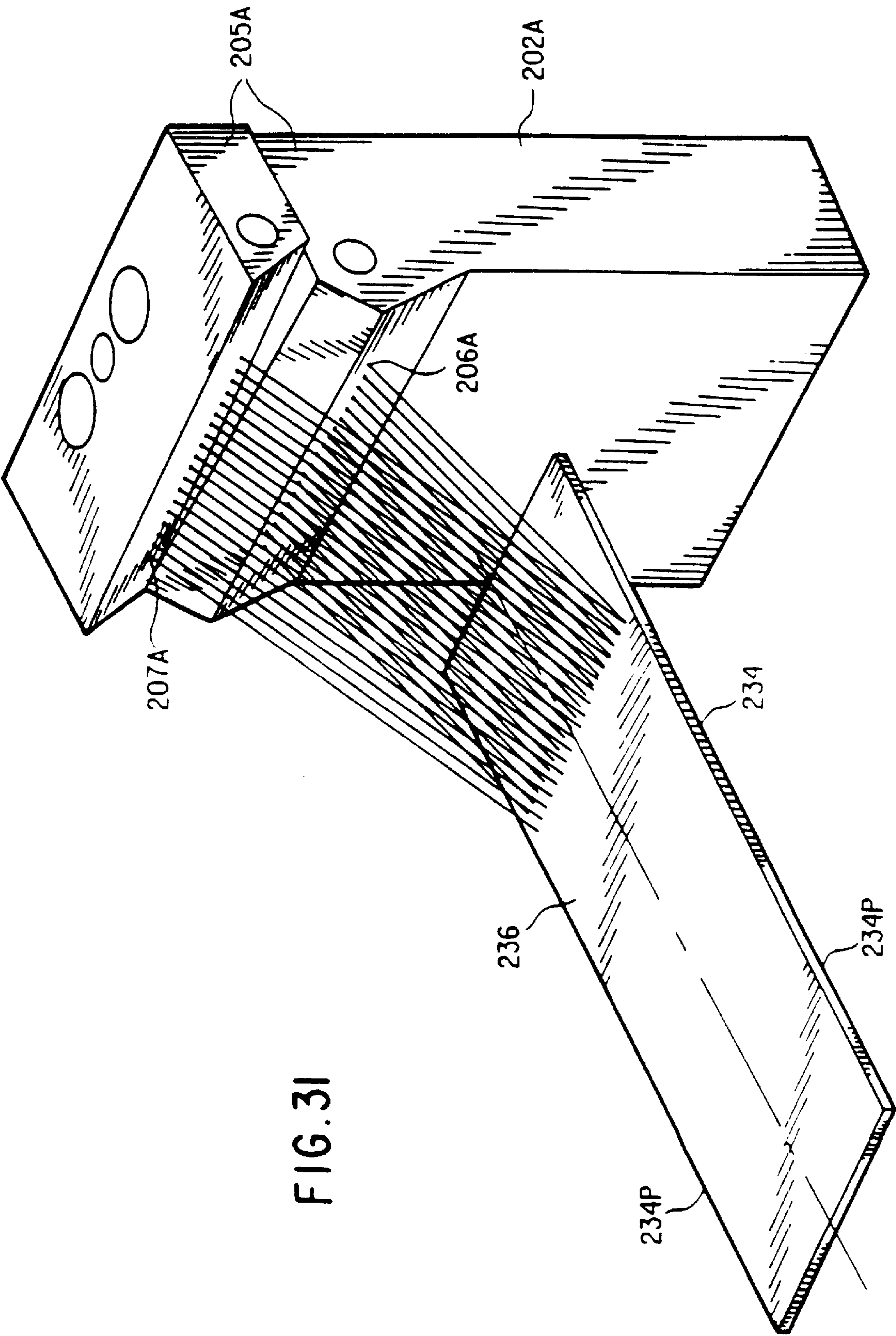


FIG. 30B



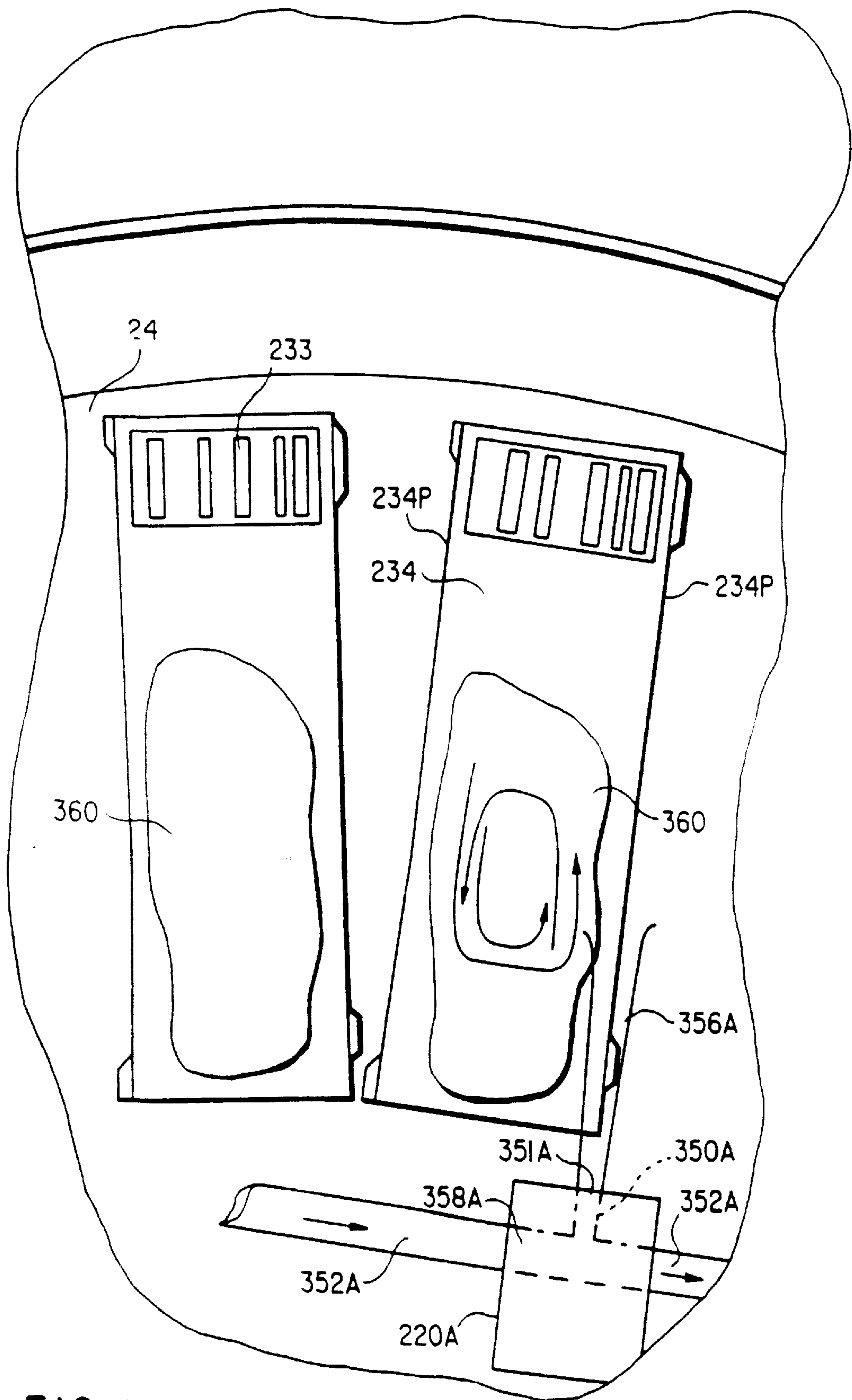


FIG. 32

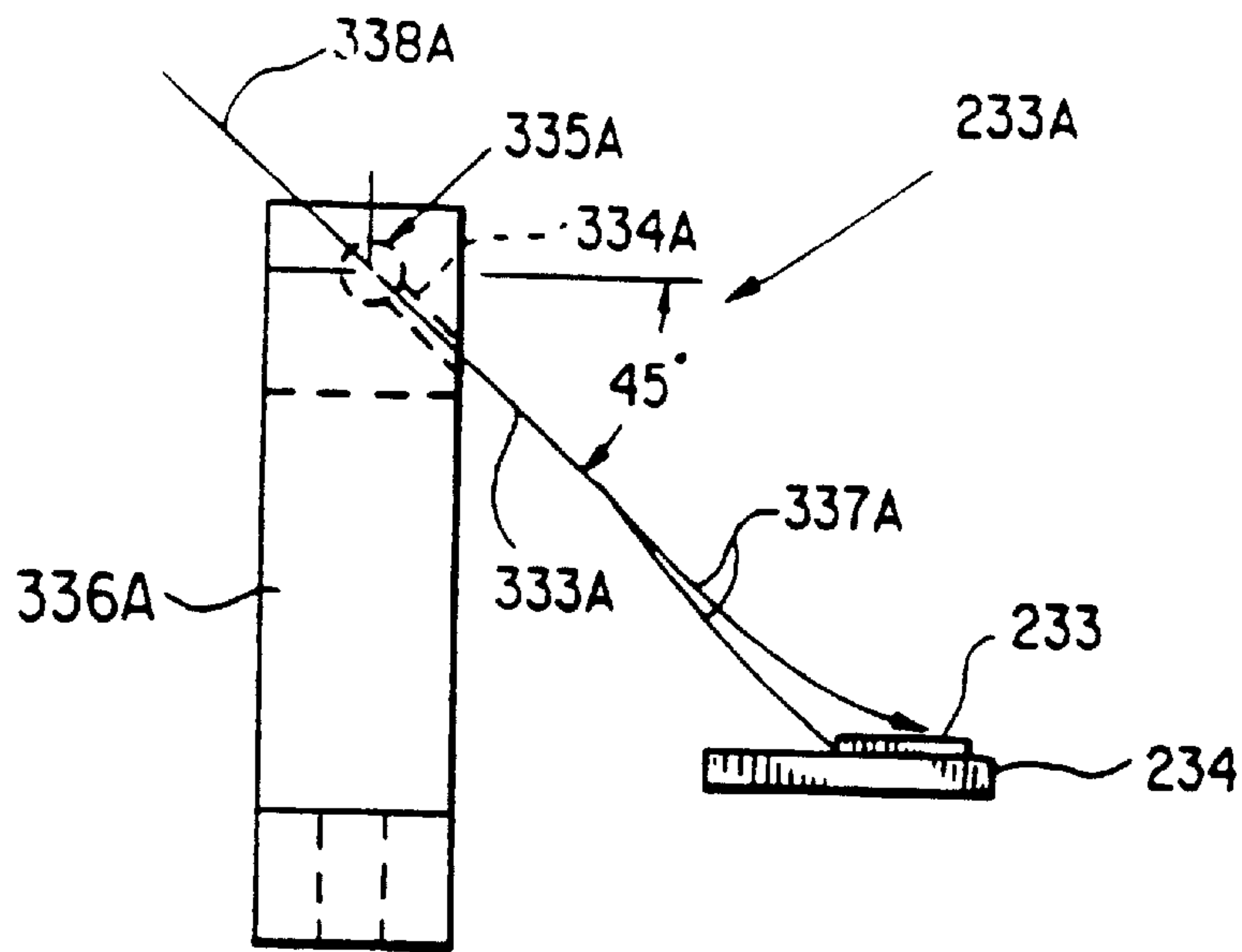


FIG. 33A

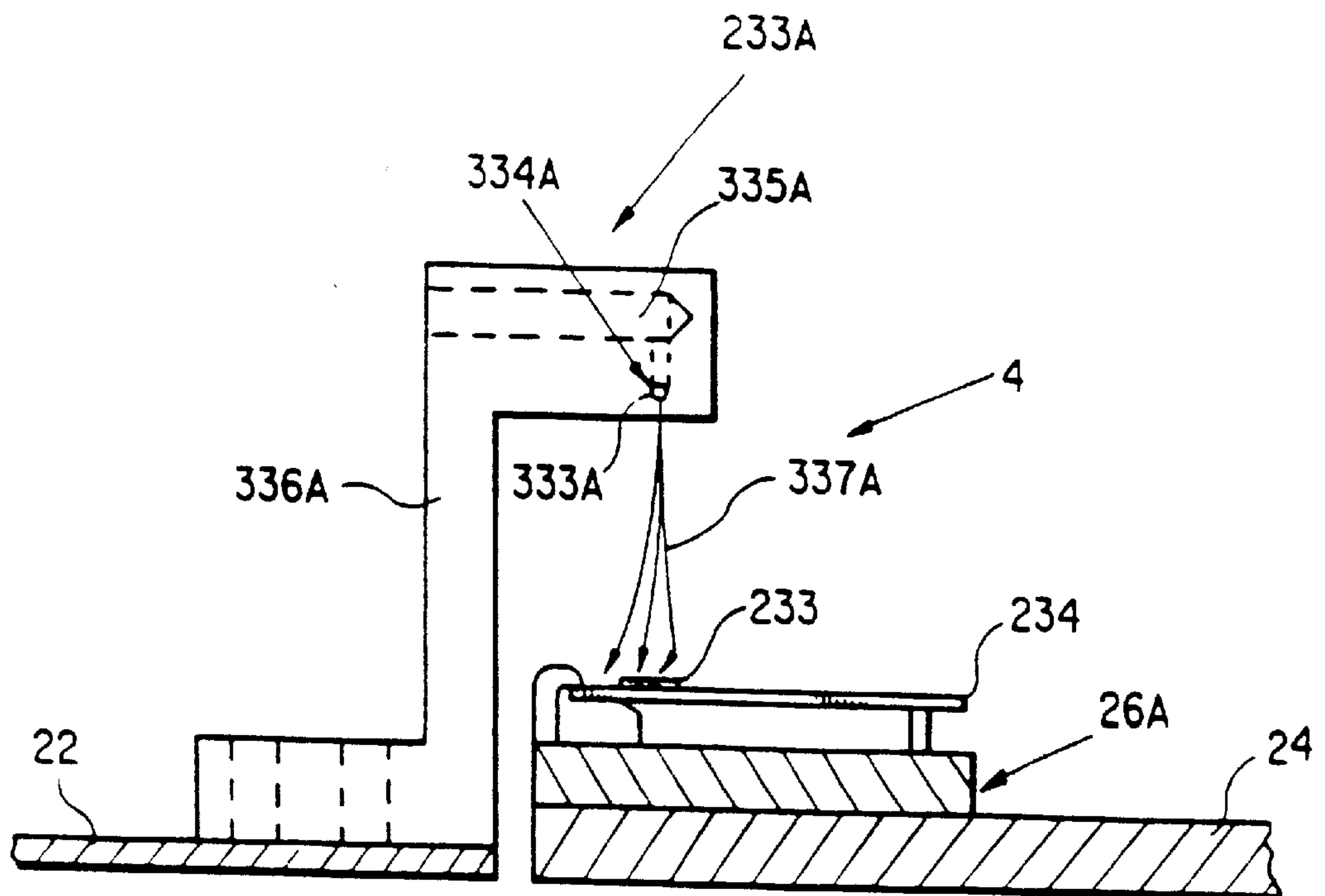


FIG. 33B

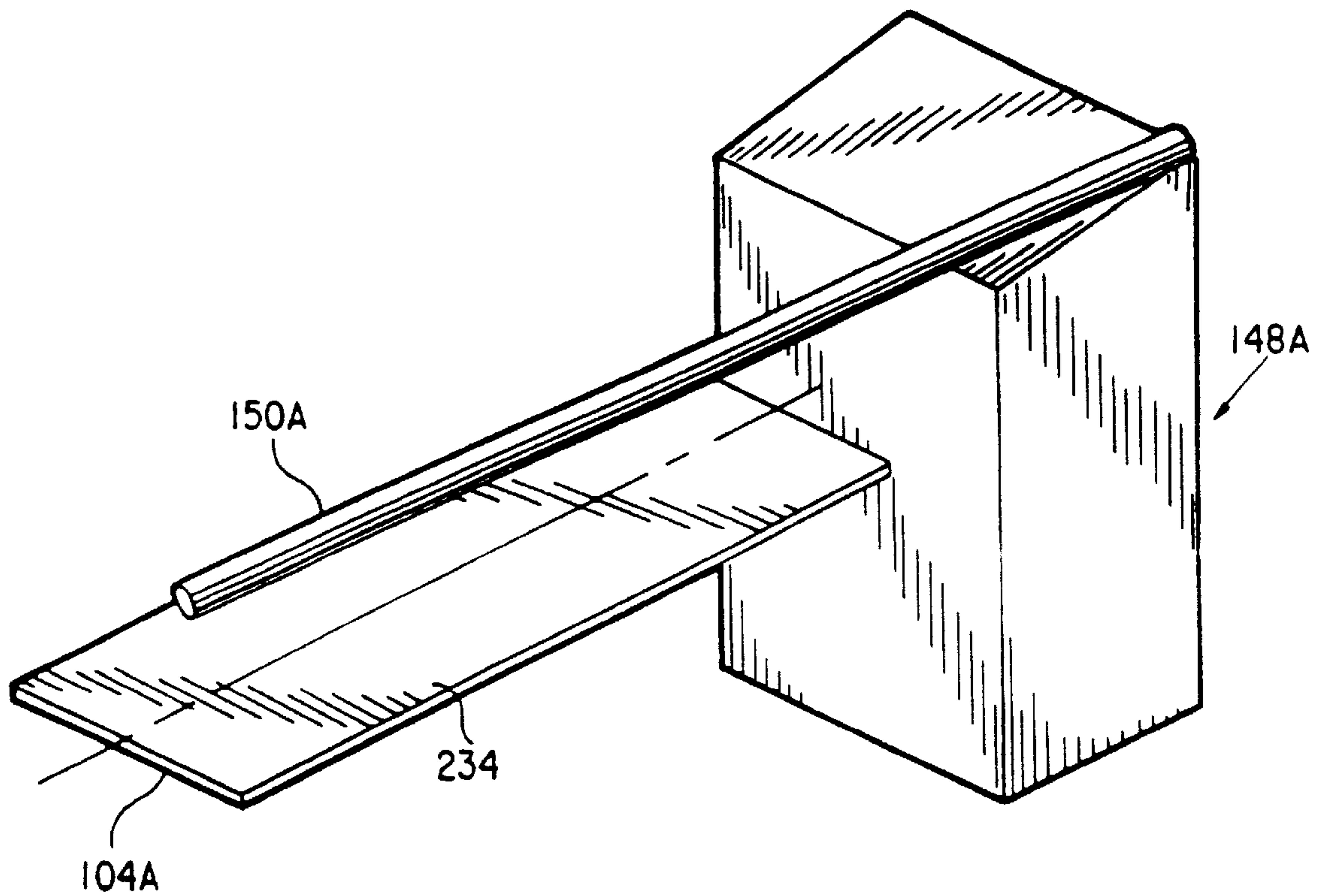


FIG. 34

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AUTOMATED BIOLOGICAL REACTION
APPARATUS

This is a continuation of application Ser. No. 08/906,678, filed Aug. 5, 1997, pending, which is a continuation of application Ser. No. 08/479,415, filed Jun. 6, 1995, U.S. Pat. No. 5,654,200, which is a division of application Ser. No. 08/352,966, filed Dec. 9, 1994, U.S. Pat. No. 5,595,707, which is a continuation of application Ser. No. 07/924,052, filed Aug. 31, 1992, abandoned, which is a continuation-in-part of application Ser. No. 07/488,601, filed Mar. 2, 1990, abandoned.

TECHNICAL FIELD

This invention relates an improved biological reaction platform which can be used for a wide variety of assays, for example, automatic immunostaining of tissue sections, in situ DNA analysis, immunoassays such as ELISA, and the like. The automatic device of this invention can be used to process a large number of samples such as tissue sections mounted on slide surfaces using agents and protocols pre-selected by the operator, while maintaining the slide surfaces in a substantially horizontal plane throughout the incubation cycles.

BACKGROUND ART

Immunostaining and in situ DNA analysis are useful tools in histological diagnosis and the study of tissue morphology. Immunostaining relies on the specific binding affinity of antibodies with epitopes in tissue samples, and the increasing availability of antibodies which bind specifically with unique epitopes present only in certain types of diseased cellular tissue. Immunostaining requires a series of treatment steps conducted on a tissue section mounted on a glass slide to highlight by selective staining certain morphological indicators of disease states. Typical steps include pretreatment of the tissue section to reduce non-specific binding, antibody treatment and incubation, enzyme labeled secondary antibody treatment and incubation, substrate reaction with the enzyme to produce a fluorophore or chromophore highlighting areas of the tissue section having epitopes binding with the antibody, counterstaining, and the like. Each of these steps is separated by multiple rinse steps to remove unreacted residual reagent from the prior step. Incubations are conducted at elevated temperatures, usually around 40° C., and the tissue must be continuously protected from dehydration. In situ DNA analysis relies upon the specific binding affinity of probes with unique nucleotide sequences in cell or tissue samples and similarly involves a series of process steps, with a variety of reagents and process temperature requirements.

Automated systems have been explored to introduce cost savings, uniformity of slide preparation, and reduction of procedural human errors. Stross, W. et al, *J.Clin.Pathol.* 42:106–112 (1989) describes a system comprising a series of baths positioned under the circumference of a circular, rotatable disc from which slide trays are suspended. The disc is lifted to lift slide trays from their baths, turned to position the slide trays above the next consecutive bath, and lowered to immerse the slide trays in the baths. This operation can be automated with suitable timers and switches. This system exposes each of the slides to the same treatment and relies on dipping for application of reactants and rinsing.

Stark, E. et al, *J.Immunol.Methods.* 107:89–92 (1988) describes a microprocessor controlled system including a revolving table or carousel supporting radially positioned

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slides. A stepper motor rotates the table, placing each slide under one of the stationary syringes positioned above the slides. A predetermined volume of liquid, determined by a dial, is delivered to a slide from each syringe. Microprocessor controls are provided.

Cosgrove, R. et al, *ACL*, pp 23–27 (December, 1989) describe an immunostaining apparatus for auto-pipetting reagents into a slide well from a carousel holding up to 18 reagent vials. Below each well, a coverplate spaced from the surface of each slide provides cover and defines a reagent flow channel. The slides are suspended at a steep angle. Reagent from the well flows downward over the slide surface. A row of slides are suspended for sequential treatment. Washing is accomplished by a 3 to 4 minute continuous running wash over the sample, yielding an estimated 20:1 wash/reagent ratio.

Brigati, D. et al, *J.Histotechnology* 11:165–183 (1988) and Unger, E., Brigati, D. et al, et al, *J.Histotechnology*, 11:253–258 (1988) describe the Fisher automated workstation using capillary gap technology. A coverplate is placed over the slide, forming a capillary gap. Liquid is introduced into the capillary gap by placing the lower edge of the plate-slide pair in a liquid. Liquid is removed by placing the lower edge of the plate-slide pair on a blotter. The system is further described in U.S. Pat. Nos. 4,777,020, 4,798,706 and 4,801,431. The previously known devices are limited in their performance and unable to satisfy the needs for automated, high precision immunohistology.

It is an object of this invention to provide a device which provides more rapid, reliable and more reproducible results than standard methods; can perform any standard immunochemical assay including assays relying on immunofluorescence, indirect immunoassay procedures, peroxidase anti-peroxidase methods, or avidin-biotin technology; preforms all steps of the immunohistochemical assay irrespective of complexity or their order, at the time and temperature, and in the environment needed; and is cost effective in terms of equipment, reagent and labor costs.

DISCLOSURE OF THE INVENTION

The automated biological processing apparatus of this invention comprises a reagent carousel cooperating with a sample support carousel to apply a sequence of preselected reagents to each of the samples with interposed mixing, incubating, and rinsing steps cooperating therewith. The slide support carousel has a plurality of slide supports thereon and drive means engaging the slide support carousel for consecutively positioning each of a plurality of slide supports in a reagent receiving zone. The reagent carousel has a plurality of reagent container supports thereon and drive means engaging the reagent carousel for rotating this carousel and positioning a preselected reagent container support and associated reagent container in a reagent supply zone. The apparatus has a reagent delivery actuator means positioned for engaging a reagent container positioned on a container support in the reagent supply zone and initiating reagent delivery from the reagent container to a slide supported on a slide support in the reagent receiving zone.

The apparatus preferably has bar code readers positioned to read bar codes on the sample containers or slides and on the reagent containers. Each of the carousels have homing systems containing a detectable component and a proximity detector therefor for indexing the position of the reagent containers and slides.

One particular advantageous feature of the present invention is that by employing a computer control arrangement to

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control the positioning of the reagent and slide support carousel, different reagent treatments can be individually performed for each of the various tissue samples by appropriate programming of the apparatus. Additionally, the provision of the bar code readers permits tracking of each of the tissue samples as well as a record of the reagents applied thereto.

The apparatus preferably has a heating chamber means surrounding the slide support carousel for heating slides supported thereon to a predetermined temperature. The heating chamber means includes a hot gas manifold having a plurality of hot gas outlets positioned above the slide supports. The heating chamber means includes a temperature sensor and a hot gas control means connected to the temperature sensor for increasing heat supplied to gas flowing through the manifold and for increasing the hot gas flow rate if further heat is required to maintain the heating chamber at a preselected temperature. The temperature sensor is a thermistor, the tip thereof being enclosed in a heat sensitivity reducing jacket. The hot gas control system includes two heating components with separate controls and a speed control for the hot gas fan.

The drive means engaging the slide support carousel is also a means for consecutively positioning each of a plurality of slide supports at rinse zone, an evaporation control liquid and reagent receiving zone, a vortex mixing zone including vortex mixing means, and an incubation zone formed by the heating chamber means.

According to a first embodiment of the rinse zone, rinse spray means are positioned adjacent to the rinse zone for applying pulses of rinse liquid to the surface of each of the slides positioned in the rinse zone. The apparatus slide supports are, according to this first embodiment of the rinse zone, pivotally mounted for pivotal motion from a horizontal slide incubation position to a tilted slide draining position following each pulse of rinse liquid.

According to a second embodiment of the rinse zone, first and second rinse spray means are respectively positioned only at the beginning and end of the rinse zone, so as to be spaced from one another. The first rinse spray means deposits a layer of rinse liquid onto a slide upon entering the rinse zone and the second spray means, after a predetermined waiting period, uses pulsed streams of rinse liquid, alternately directed at the longitudinal edges of the slides, to knock the previously deposited layer of rinse liquid off of the slide as the slide exits the rinse zone. According to this second embodiment of the rinse zone, the apparatus slide supports are stationary, a jet drain being provided at, for example, the end of the rinse zone, which directs a stream of fluid, such as, for example, air or the like, over the slide to drain any remaining rinse liquid off of the slide surface.

The apparatus preferably has a volumetric pump means, and a reagent delivery actuator means positioned for activating the volumetric pump means, thereby effecting delivery of reagent from a reagent container by the volumetric pump to the reagent delivery zone. An evaporation inhibiting liquid application means is positioned adjacent the reagent delivery zone.

Vortex agitation means are positioned adjacent the agitation zone for stirring reactants on a slide supported in the vortex agitation zone.

The pivoting slide support has distal and proximal ends, the distal end having raised terminal and lateral distal guide tabs with guide termini. The proximal end has first and second lateral guide tabs with opposed slide engaging surfaces for engaging and holding the lateral edges of a slide.

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The guide termini are lower than the upper slide surface plane. In this embodiment of the slide support, the slide support surface is tipped or pivoted by a tipper to drain rinse liquid from the surface of the slide.

The stationary slide support has a slide support platform at a proximal end and a slide support post at a distal end thereof. The distal end also has raised lateral distal guide tabs with guide termini between which a slide is positioned. The slide support platform at the proximal end has a guide edge and a slide clamping arrangement for clamping a slide to the support platform without interfering with the reading operation of the bar code reader. The distal guide termini are lower than the upper slide surface plane to prevent wick-off of liquid on the slide surface. In this embodiment, rinse liquid is drained from the surface of the slide employing a jet drain which directs a stream of fluid, i.e., gas or liquid, over the slide surface.

An improved biochemical method of this invention with increased sample dehydration protection comprises carrying out a biochemical reaction under a layer of evaporation inhibiting liquid. The improvement comprises (a) covering the sample with an aqueous surface layer by applying an aqueous solution to a planar support surface adjacent a biological sample mounted thereon; and (b) covering the aqueous surface layer with an evaporation inhibiting liquid layer by applying the evaporation inhibiting liquid to the planar support surface adjacent the biological sample in an amount sufficient to form a continuous layer of evaporation inhibiting liquid over the sample. The evaporation inhibiting liquid is substantially water-insoluble, substantially water-immiscible and substantially non-viscous; has a specific gravity less than water, and a boiling point above 50° C.; and is devoid of chemical characteristics which would significantly interfere with biochemical reactions carried out on the sample. The biological sample can then be optionally treated (c) with an aqueous reagent solution by applying the reagent solution to the planar support surface adjacent the biological sample. The reagent solution flows to the biological sample under the evaporation inhibiting liquid layer, and the sample is continuously protected from dehydration by the evaporation inhibiting layer.

In another aspect of this invention, the reagent solution is stirred on the surface of the biological sample by applying at least one gas stream to an area of the surface of the evaporation inhibiting liquid layer between the center of the evaporation inhibiting layer and the edge of the planar support surface, the gas stream having a central axis forming an acute angle with the planar support surface. According to one embodiment of the present invention, the reagent solution is preferable stirred by a vortex formed by applying two off-center gas streams, flowing in opposite directions, to the surface of the evaporation inhibiting liquid layer. According to a further embodiment of the present invention, the reagent solution is stirred by a vortex formed by applying a single gas stream along a longitudinal edge of the slide, the gas stream originating from the distal edge of the slide.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a left front, isometric view of the automated immunostaining apparatus according to a first embodiment of this invention which employs a tipper rinse method, with the cabinet shell removed.

FIG. 2 is an exploded right front isometric view of the apparatus shown in FIG. 1.

FIG. 3 is a partial exploded left front isometric view of the apparatus shown in FIG. 1.

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FIG. 4 is a partial exploded right rear isometric view of the apparatus shown in FIG. 1.

FIG. 5 is a top view of a pivotally mounted slide support.

FIG. 6 is an isometric view of the underside of the slide support component.

FIG. 7 is a side view of the pivotally mounted slide support of FIG. 5 showing the tipper and mounting details.

FIG. 8 is an isometric view of the mounted slide support of FIG. 7 in the untipped position.

FIG. 9 is an isometric view of the mounted slide support of FIG. 7 in the tipped position.

FIG. 10 is a distal end view of the mounted slide support in the tipped position.

FIG. 11 a fragmentary top view of the slide support carousel showing details of the slide treatment stations.

FIG. 12 is a schematic cross-sectional view of a rinse station taken along the line A—A in FIG. 11, showing details of rinse liquid flow on a slide.

FIG. 13 is a top schematic view of the rinse stations showing details of the rinse liquid distribution on slides being treated therein.

FIG. 14 is an isometric view of the slide treatment bar code reading, rinse, reagent receiving and vortex mixing stations.

FIG. 15 is a schematic, fragmentary cross-sectional view of the evaporation inhibiting liquid and reagent receiving station, taken along the line B—B in FIG. 11.

FIG. 16 is a cross-sectional view of the vortex mixing assembly, taken along the line C—C in FIG. 11.

FIG. 17 is a top schematic view of the vortex mixing zone, showing details of the vortex mixing action.

FIG. 18 is a schematic representational cross-sectional view of a slide following the rinse liquid, evaporation inhibitor and reagent application steps.

FIGS. 19A–19B are cross-sectional views of respective alternative embodiments of a rinse liquid container and associated heating components.

FIG. 20A is a bottom, isometric view of one embodiment of a reagent container support tray.

FIGS. 20B–20C are side sectional views of a further embodiment of the reagent container support tray.

FIG. 21 is a fragmentary cross-sectional view taken along the line D—D in FIG. 11 showing the slide carousel metal proximity sensor indexing system of this invention.

FIG. 22 is a schematic view of the pneumatic system of the automated immunostaining apparatus of this invention.

FIG. 23 is a schematic drawing of the 120 volt AC power distribution in the apparatus of this invention.

FIG. 24 is a schematic drawing of the DC power distribution in the apparatus of this invention.

FIG. 25 is a schematic drawing of a first portion of the computer digital I/O system in the apparatus of this invention.

FIG. 26 is a schematic drawing of a second portion of the computer digital I/O system in the apparatus of this invention.

FIG. 27 is schematic drawing of the computer serial and floppy disk I/O system in the apparatus of this invention.

FIG. 28 is a further embodiment of the intermediate section of the apparatus of this invention which dispenses with the tipper rinse method.

FIGS. 29A–29B are top and side views respective an alternative embodiment of the slide support for use with the embodiment of FIG. 28.

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FIG. 30A is a side, isometric view of one embodiment of a single wash block nozzle for use with the embodiment of FIG. 28.

FIG. 30B is a side, cross-sectional view of the single wash block nozzle of FIG. 30A.

FIG. 31 is a side, isometric view of one embodiment of a dual wash block nozzle for use with the embodiment of FIG. 28.

FIG. 32 is a top view of a further embodiment of the vortex mixers for use with the embodiment of FIG. 28.

FIGS. 33A–33B are side and front views respectively of bar code cleaning arrangement for use with the embodiment of FIG. 28.

FIG. 34 is a schematic of a jet drain for draining liquid from an upper surface of a slide.

BEST MODE FOR CARRYING OUT THE INVENTION

The automated immunostaining apparatus of this invention preforms all steps of immunohistochemical and in situ DNA assays irrespective of complexity or their order, at the time and temperature, and in the environment needed. Specially prepared slides containing a bar code identifier and a mounted tissue section are placed in special support on a carousel, subjected to a preprogrammed sequence of reactions, and are removed from the carousel, ready for coverslipping and histological examination. For purposes of clarity of the following description of the apparatus of this invention and not by way of limitation, the apparatus will be described in terms of immunohistochemical processes.

FIG. 1 is a front right, isometric view of the automated immunostaining apparatus of this invention, with the cabinet shell removed. Liquid and air supply tubing and electrical wiring connecting the respective components are conventional, well known in the art, and are omitted from the drawings for purposes of clarity. The apparatus has an upper section 2, intermediate section 4 and lower section 6. In the upper section 2, reagent bottle support carousel 10 is mounted for rotation about its central axis 7 on upper support plate 8. Reagent bottles 12 required for the immunohistochemical reactions to be conducted during slide treatment cycle are supported by the carousel 10, mounted in reagent bottle receptors 11. These receptors 11 are configured to receive volumetric pump outlet tube 307, shown in detail in FIG. 15. The receptors 11 are preferably equally spaced in a circular pattern axially concentric with the carousel axis 7. The number of receptors 11 provided should be sufficient to accommodate the number of different reagent bottles 12 required for a cycle or series of cycles. Twenty-five receptors 11 are shown, but the number can be smaller or greater, and the diameter of the carousel 10 can be increased to accept a larger number of reagent bottles 12. The carousel 10 is rotated by the stepper motor 14 drive belt 16 to a position placing a selected reagent bottle 12 in the reagent deliver position under the air cylinder reagent delivery actuator 18 over a slide to be treated with reagent. Reagent tray motor driver 20 is connected to stepper motor 14.

The intermediate section 4 comprises support plate 22 upon which the slide support carousel 24 is rotatably mounted. The carousel 24 supports slide supports 26. Heated air supply chamber 28 communicates with the heated air supply manifold 30 supported on the underside of plate 8 and lid heated air supply manifold 31 mounted on the upper plate 8 by hinged supports 33. The support plate 22 also supports the conventional computer board 32, LCD display

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34, disk drive 35 and computer 36 used to operate the apparatus. Air pressure regulator 38, as best seen in FIG. 2, regulates the pressure of air delivered to the evaporation inhibitor and rinse liquid delivery systems described in FIG. 22.

The lower section 6 includes support plate 40 upon which are supported accessories such as power supply filter 42 and hot water supply 44.

FIG. 2, FIG. 3 and FIG. 4 are exploded right front, left front and right rear isometric views of the apparatus shown in FIG. 1. Tipper air cylinders 46 are positioned on support plate 8. These cylinders are aligned to actuate a tipper cam surface 148 against a slide support tab surface 112 shown in detail in FIGS. 8, 9 and 10.

In the intermediate section 4, the stepper motor 48 rotates the slide support carousel 24, engaging drive belt 25 (FIGS. 3 and 4) engaging the perimeter of the slide support carousel 24. Splash guard 50 is a wall which surrounds the sides, back and part of the front of the carousel 24, defines the heating zone and contains the liquid spray and droplets produced in the processing. It extends upward from the intermediate plate 22 to a position adjacent the upper plate 8, leaving an air flow gap between the upper edge of the splash guard 50 and the underside of the plate 8. Mounted on the underside of upper support plate 8 above the carousel 24 and within the perimeter of the splash guard 50 is the heated gas supply manifold 30 (FIG. 2). Heated air is directed downward and over the slide supports 26 by holes 336 (FIG. 15) in the manifold 30. The heated air then passes upward over the top of the splash guard 50 and exits the device. Extending upward through central opening 52 of carousel 24 into the heated air supply chamber 28 is the fan shroud 54 and axially positioned fan 56. The fan 56 is positioned over air vents 57 in the bottom plate 22. The annular waste liquid sump 58 surrounds the shroud 54, below liquid outlet ports 292 (FIG. 14), and is supported on the bottom of plate 22. The waste reagent and rinse liquids are collected in the sump and passed to a drain through an outlet tube in the sump bottom (not shown).

Rinse and liquid coverslip spray blocks 60 are supplied with liquid through conventional solenoid valves 62.

Temperature controller 66, mounted on support plate 22, controls the heat energy supplied to the heated water container 44. Temperature controllers 68 and 70, mounted on support plate 40 (FIG. 4), control the temperature of the air in the heated air supply chamber 28 by controlling energy supplied to respective annular heater elements 331 and 332 (FIG. 15). Slide carousel stepper motor driver 72 and relay 74 operate stepper motor 48. Power supplies 76 and 78 provide power to the stepper motors and control systems. Air compressor 80 supplies air to the air filter 82 and air pressure regulators 38, 64 and 86.

FIG. 5 is a top view of a first embodiment of a mounted slide support 26 with slide edges 100 and 101 represented by dashed lines. The slide support 26 has a support plate 102 with a distal end 103 and a proximal end 104. The distal end 103 has a raised terminal guide end tab 106 and two lateral guide tabs 108 and 110 with the upper edges constituting guide tab termini. The distance between the upper surface of the slide support 26 and the guide tab termini (the elevation above the upper surface) is less than the thickness of a conventional microscope slide. The proximal end 104 of the slide support 26 has opposed lateral guides 112 and 114 for engaging the lateral edges of a slide and a terminal end tab 115 for engaging the proximal end of a slide. The proximal end 104 of the slide support 26 has an inflexible support

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portion 116 providing a lateral edge 120 and a flexible arm 118 including a lateral edge 122 positioned such that lateral edges 120 and 122 oppose one another. The distance between the slide edge engaging surfaces 111 and 113 of the guide tabs 112 and 114 is less than the width of a slide to be supported on the slide support 26. A standard slide has a width of 1 inch or 25 mm, and the preferred distance between the slide edge engaging surfaces 111, 113 of the tabs 112, 114 for supporting a standard slide is from 20 to 24 mm. The flexure of arm 118 permits positioning of the slide between the lateral guide tabs and terminal end tabs 106, 115. The distance between the opposing tab surfaces 111 and 113 causes the slide support 26 to apply a positive pressure on the edges of a slide, retaining the slide securely on the slide support 26 during the tilting and other processing steps. The upper surface of the support plate 102 is preferably planar and smooth so the wet slide rests closely on the surface 102, and surface tension will resist vertical movement of the slide from the support surface 102.

FIG. 6 is an isometric view of the underside of the slide support 26. The inflexible portion 116 has an integral pivot support 124 which reinforces the inflexible portion 116 to prevent flexure. The flexible arm 118 has sufficient depth or thickness to limit the flexural movement of the arm 118 to a horizontal direction. This insures effective cooperation and pressure between the guide tab 112 on the inflexible portion 116 and the guide tab 114 on the flexible arm 118 to assist in retaining the slide in place on the slide support 26 during the tipping operation described in detail hereinafter.

FIG. 7 is a side view of a mounted slide support showing the tipper and mounting details. The upper pivot support 124 is pivotally mounted on the lower pivot support 126. Lower pivot support 126 has upward extending projections 128 and 130 which engage the ends 132 and 134 of the upper pivot support 124. Pivot pin 136 extends through an axially aligned hole in projection 128 into an axially aligned receptor hole 138 (FIG. 6) in the opposing end 132 of the upper pivot support 124. At the opposite end, axially concentric with pivot pin 136, pivot pin 140 extends through a hole in projection 128 (not shown) into a respective receptor hole in the opposing end 134 of the upper pivot support 124. The slide support 102 is thus mounted for pivotal motion around the common pivot axis of the pins 136 and 140. Bias spring 142 is supported on pin 134, one end 141 pressing against the lower abutment surface 143 of the inflexible support portion 116, and the other end 144 bearing against spring stop groove 145 in the spring stop 146. The tip 148 of tipper 150 is positioned above the upper surface of guide tab 112 when the slides are positioned in a rinse station, described in greater detail hereinafter with respect to FIG. 13.

The pivot pins 136 and 140 support the upper surface of the slide support 102 at a small angle 'a' from the horizontal plane to aid liquid flow toward the distal end 103 during treatment. Angle 'a' is preferably in the range of from 0.3 to 1.0°. The upper surface 151 of the inflexible support portion 116 and the upper slide surface 152 (dotted line) supported thereon are thus maintained at a slight incline from the horizontal plane downward toward the distal end 103 of the slide support 26.

FIG. 8 is an isometric view of a slide (dashed lines) mounted on slide support 26 in the untipped position, FIG. 9 is an isometric view of the mounted slide support 26 in the tipped position, and FIG. 10 is a distal end view of the mounted slide support 26 in the tipped position. Vertically downward pressure of the tipper tip 148 against the upper guide tab surface 154 of guide tab 112 rotates the support plate 102 about the pivot axis 156 defined by the pivot pins

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136 and 140. The pivot axis 156 (FIG. 5) preferably lies in a vertical plane through the midpoint of distal end 103 and the left edge proximal end 104 of the slide support 26. The tipping action tilts the slide surface to an angle 'c' of approximately 60° from the vertical (FIG. 10). It sharply lowers distal corner 158 and sharply raises proximal corner 160, breaking the liquid meniscus on the slide surface and directing the liquid flow 159 to the corner 158 and off the surface of the slide into drain hole 292. The pivotal movement increases the pressure of the spring 142 against spring stop groove 145, and as the tipper 150 is raised, the slide support 25 returns to its original position. The slide support return pivot motion is terminated when distal corner 162 of the support plate 102 abuts stop surface 164 of the lower pivot support 126.

FIG. 11 a fragmentary top view of the slide support carousel 24 showing details of the various slide treatment stations. Rinse nozzle blocks 200, 202 and 204 and the adjacent respective slides 206, 208 and 210 define successive rinse zones, details of which are shown in FIGS. 12–14. Evaporation inhibitor liquid application block 212 and the adjacent slide 214 define the evaporation inhibitor and reagent application zone, details of which are shown in FIG. 15. Air cylinder reagent delivery actuator 18, supported by support arm 216, contacts reagent bottle 218, directly over slide 214. Vortex mixer air jet blocks 220, 222 and 224 are positioned adjacent slides 226 and 228 in the agitation zone, details of which are shown in FIG. 16 and 17. The hanger 352 is mounted on the tip of blocks 220 and 222 and supports suspended block 224. Pressurized air is delivered to block 224 by conduit 358. As the slide support carousel 24 positions each slide for successive treatment in the rinse zones, evaporation inhibitor and reagent application zone, and agitation zones (counter-clockwise movement of the carousel), the tissue sections on each slide are first rinsed and then covered with evaporation inhibitor. Reagent is applied from a preselected reagent bottle to the tissue through the evaporation inhibitor layer, and the reagent is agitated through the evaporator inhibitor layer by the vortex mixer. Each slide then is moved around the incubation zone, a circular path traveled by the slide support carousel 24, heated with hot air from the heated air manifold 30, and the reagent reacts with the sample. As the carousel 24 continues to increment around the circle, each slide is returned to the rinse stations, etc, for application of the next reagent required in the reaction. This entirely automated progress continues until the desired reactions are completed.

Bar code reader 231 (FIG. 14) above slide 205 reads a slide bar code 233 (FIGS. 13 and 17) on each slide. The slide bar codes 233 identifies the slide sample and the particular immunohistochemical process required for that sample. This information is fed into the computer and correlated with the indexed position of that slide with respect to "home", to control the sequence of reagent chemicals to be applied to that slide in the reagent application zone.

FIG. 12 is a schematic cross-sectional view of a rinse station taken along the line A—A in FIG. 10, showing details of rinse liquid flow on a slide. Rinse block 200 mounted on plate 22 has a heated rinse liquid supply channel 230 communicating with rinse liquid nozzle 232. The slide 234 has a sloping surface at an angle 'a', being supported on the sloping surface of the slide support 102. The slide 234 has a rinse liquid impact zone 236 adjacent the proximal end 104 between the bar code 233 and the sample 238. The impact zone 236 is at a higher elevation than the tissue section 238 supported adjacent the distal end 103. The nozzle axis 240 has an angle 'b' which directs liquid against the slide surface

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impact zone 236. The impact zone 236 is above the tissue section 238 on the sloped surface of slide 240, and the rinse liquid stream 242 flows across the upper surface of the tissue section 238 toward the distal end 103. The angle 'b' preferably has an angle of from 15 to 35°, and the distance between the exit of nozzle 232 and the slide 124 is selected to direct the rinse liquid precisely on the impact zone 236, avoiding disturbance of the fragile tissue section 238.

The slide support carousel 24 is rotated above the plate 22, the outer periphery being supported by low friction slide bearings 244 arrayed in an axially concentric circular path on plate 22 under the outer periphery of carousel 24.

FIG. 13 is a top schematic view of one embodiment of the rinse stations showing details of the rinse liquid distribution on slides being rinsed therein. Slides 234, 246, and 248 are positioned in the path of heated rinse solutions (dotted lines) from rinse station blocks 200, 202 and 204. Fragile tissue sections 238, 250 and 252 are positioned adjacent the distal end of the slides. The rinse liquid impact zones 236, 254 and 256 are positioned between the tissue sections and the proximal ends of the slides, to avoid direct impact of the liquid jets from the rinse block nozzles. The rinse nozzles on each block are preferably 11.5 mm apart. Rinse block 200 has right offset nozzles 232 and 258 (offset 2 mm to the right of center) supplied by channel 230 connected to supply tubing 260. This directs the rinse fluid toward the right surface of the slide, effecting a transverse flow path across the tissue section 238 to the distal end drain corner 158. Rinse block 202 has symmetrical nozzles 262 and 264 supplied by channel 266 connected to supply tubing 268. The symmetrical nozzle configuration effects a central flow path across the tissue section 250. Rinse block 204 has left offset nozzles 270 and 272 (offset 2 mm to the left of center) supplied by channel 274 connected to supply tubing 276. The left offset nozzles 270 and 272 direct a rinse flow path down the left side of the tissue section 252. The nozzle patterns provide effective rinse solution flow distribution across all portions of the tissue section surface as the slide is treated in each successive rinse section.

FIG. 14 is an isometric view of the rinse stations, a evaporation inhibiting liquid and reagent application station, and agitation stations, showing details of the slide tipping action in the rinse sections. Tipper air cylinders 46 (FIG. 3 and 4) comprises three conventional air cylinders 278, 280 and 282 with internal pressurized air activated pistons or equivalent actuators. Pressurized air delivery to the cylinders causes respective tipper tips 148, 284 and 286 to move downward, pressing against respective slide support tabs 112, 288 and 290. Three tipper positions are shown to illustrate the action thereof. Tipper tip 148 is shown in the fully withdrawn or resting position, and slide 206 is in the rinse solution receiving position. After application of heated rinse solution, the tipper descends through an intermediate position shown by tipper tip 284 and slide support 208, to the drain position shown by tipper tip 286 and slide support 210. Liquid drains from the left distal corner (lowest corner) into a drain hole 292.

In each rinse station, the sample is treated with a repeated, preferably at least seven, rinse cycles. Each rinse cycle comprises application of approximately 500 μ L of heated rinse solution in a short pulse (120 msec) to the slide, followed by tipping the slide to drain away the rinse solution. An estimated 150 μ L of liquid remains on the slide after draining. These rinse cycles are repeated in each rinse station. The short rinse pulse followed by draining prevents the formation of an equilibrium solute boundary layer and greatly increases the rinse efficiency, overcoming the bound-

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ary problems present in the prior art rinse methods. Assuming that 150 μ L of rinse solution is left after each draining step, a 23 percent dilution is achieved with each rinse cycle. Thus the effective dilution in the combination of the three rinse stations is estimated to be 0.2 parts per trillion, many orders of magnitude more effective than prior art, biochemical rinse procedures. This greatly increases the sensitivity of the immunohistological process.

FIG. 15 is a schematic, fragmentary cross-sectional view of the evaporation inhibiting liquid and reagent application station, taken along the line B—B in FIG. 11. Evaporation inhibitor liquid distributor block 212 has a supply channel 293 and outlet nozzles 294.

The evaporation inhibiting liquid is substantially water-insoluble, substantially water-immiscible and substantially thin or non-viscous. It has a specific gravity less than water, and a boiling point above the process temperature, preferably above 100° C. It should be devoid of chemical characteristics which would significantly interfere with biochemical reactions carried out on the sample, that is, the reactions taking place between the reagents and tissue sample on the slide. Preferred evaporation inhibiting liquids are hydrocarbons, optimally non-aromatic saturated hydrocarbons, having from 9 to 18 carbons, most optimally having about 10 to 14 carbon atoms.

A small quantity of evaporation inhibitor liquid is directed by nozzle 294 in a inhibitor liquid stream 296 to an impact zone 298 on the slide between the tissue sample 238 and the proximal end 100 of the slide, so that the tissue sample is not disturbed. The evaporation inhibitor liquid flows across the surface of the water layer on the wetted tissue, forming a thin evaporation inhibiting film 299 over the aqueous layer which usually covers most of the upper surface of the slide. The tissue is now ready for application of reagent.

The reagent delivery combination includes a conventional air cylinder 18 or equivalent actuator having an internal pressurized air activated piston. It is supplied with pressurized air by tubing 300. Air cylinder 18 is supported by plate 216 and post 302 mounted on upper plate 8. Delivery of pressurized air to the cylinder 18 causes rod 304 and its attached foot 306 to move downward against a reagent container 12 positioned in the reagent delivery zone. Downward movement of reagent container 12 causes emission of a precise volume of reagent liquid 310. Suitable volumetric pumps are available from S. A. Valois and are described in U.S. Pat. No. 4,245,967 and French patent 2,528,122.

The reagent carousel support 314 is the drive plate which supports the reagent bottle carousel 10 and rotates it about its axis to place a predetermined reagent bottle 12 in the reagent delivery zone. An axially concentric circular array of low friction slide bearings 316, mounted on the upper plate 8, are positioned under the outer edge of the reagent support carousel.

The predetermined volume of aqueous reagent 310 impacts the evaporation inhibitor surface film between the impact zone 298 and the upper edge of the tissue sample 299, passing through the film to the aqueous layer beneath the film and reaching the slide surface. The reagent then flows across the tissue sample 238 under the covering film of evaporation inhibiting liquid 299. In this sequence, immediately after leaving the rinse stations, the slide is covered with the protective film to prevent any dehydration of the tissue sample 299. The reagent solution is then applied to the protected tissue. Dehydration of the tissue section would irreversibly alter its physical and chemical characteristics and impair the immunohistochemical reactions. Dehydration

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is a constant hazard because of the constant flow of heated air over the slides required to maintain them at the desired temperature. The heated air temperature is determined by the requirements of the biochemical processes required by the process. It is slightly above 40° C., preferably about 45° C., for immunochemical reactions and can be as high as from 93 to 97° C. for in situ DNA hybridization reactions.

FIG. 15 also shows detailed elements of the heated air supply chamber 28 shown in FIG. 1. Air is moved upward into the central intake manifold chamber 330 and through annular heating coils 331 and 332 mounted on annular air passageway plate 333, to heat the air to a temperature slightly above 40° C., preferably about 45° C. A higher temperature can be provided as needed for in situ DNA hybridization procedures. The heated air passes through the outlet manifold chamber 334 and out the outlet passageways 336 in the lower plate 338. Annular, axially concentric inner and outer heated air flow control curtains 340 and 342 direct the heated air downward over the slide surface. The reagent 310 falls through manifold passageway 344 to the slide surface.

The air temperature is monitored by heat sensor 345 positioned in the path of the heated air. A preferred heat sensor is a thermistor encased in a heat sensitivity adjusting jacket 347 which reduces the sensitivity of the thermocouple and approximates the thermal mass of the slides.

A reagent bar code reader 346 can be mounted on post 302, positioned to scan a reagent bar code 348 on the reagent bottle 12. Bar code 348 identifies the contents of the reagent bottle. At the beginning of a slide treatment operation, the reagent carousel 10 is rotated past the bar code reader 346, and the bar code 348 on each reagent bottle 12 is scanned. The scanned information is fed to the computer and correlated with the indexed position of the reagent carousel 10. This information is used to rotate the reagent carousel 10 to place the correct reagent bottle 12 in the application zone for each slide treatment step for each slide.

FIG. 16 is a cross-sectional view of one embodiment of the vortex mixing assembly, taken along the line C—C in FIG. 11. Outer vortex jet block 222, mounted on plate 22, has an pressurized air supply channel 350 and nozzle 351. Nozzle hanger 352 is mounted on the top of vortex block 22 and supports suspended inner vortex air jet nozzle block 224. Channel 354 supplies nozzle 355 in block 224 with pressurized air. Nozzles 351 and 355 have central axes which form angles 'd' and 'e' of from 5 to 15° with the horizontal, directing air jets 356 and 357 toward the slide surface at the corresponding acute angles.

FIG. 17 is a top schematic view of the vortex mixing zone, showing details of the vortex mixing action. Pressurized air is supplied to the nozzle channels 350 and 354 by channel 358. The reagent solution covered by a layer 360 of evaporation inhibiting liquid 360 is stirred on the surface of the biological sample by applying at least one gas stream 356 or 357 to an area of the surface of the evaporation inhibiting liquid layer 360 between the center of the evaporation inhibiting layer 360 and the edge of the planar support surface 361 or 362 of the slide 228. The gas stream impacts the surface of the evaporation liquid surface layer 360 and moves the underlying reagent solution in a circular path on the tissue section. Preferably, the reagent solution is stirred on the surface of the biological sample by a vortex formed by applying two gas streams 356 and 347. Stream 356 is directed against a area 363 of the surface of the evaporation inhibiting liquid layer between the center of the evaporation

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inhibiting layer and the slide edge **361**. Stream **357**, in a direction opposite to the direction of stream **356**, is directed against an area **364** of the surface of the evaporation inhibiting liquid layer between the center of the evaporation inhibiting layer and the slide edge **362**. Although this method is shown with respect to an evaporation liquid inhibitor covered reagent layer, it will be readily evident that it can be applied to gently stir any liquid layer overlying a fragile substance.

FIG. **18** is a schematic representational cross-sectional view of a slide **370** following the rinse liquid, evaporation inhibitor and reagent application steps. Following the rinse stages (Stage A), the tissue section **371** mounted on slide **370** is covered with a thin residual aqueous layer **372**. Following application of the evaporation inhibitor liquid (Stage B), the aqueous layer **372** and tissue section **371** is entirely covered by a layer **373** of the evaporation inhibitor liquid. Aqueous reagent **374**, applied to the slide, flows under the evaporation inhibitor layer **373** to cover the tissue section. In the vortex mixing section (Stage C), air jets directed against the surface of the evaporation inhibitor liquid **373** move it and the reagent solution **374** thereunder in a swirling or stirring action on the surface of the fragile tissue section. This gentle stirring achieves increased interaction of reagent with the tissue section while preserving the tissue from dehydration or other damage from the air jets.

FIG. **19A** is a cross-sectional view of one embodiment of a rinse liquid container and associated heating components. The rinse liquid applied to the surface of the slides by rinse blocks **200**, **202** and **204** should have a temperature above 40° C. and is preferably about 45° C. The elevated temperature is critical for the immunochemical reactions. The rinse liquid is supplied by the hot water supply **44**. The hot water supply **44** comprises an inner container of an inert material having a low coefficient of expansion such as a pyrex bottle **382** having a threaded neck **384** to which a cap **386** is attached by threads. The container **382** is surrounded by an insulating jacket **388** of suitable insulation material such as a fiberglass layer. Between the insulating jacket **388** and the bottle **382** is a heating jacket **390** with electrical power leads **392**. A suitable heating jacket is a thick sheet of silastic rubber (polysiloxane) with embedded resistance heating coils having a combined heating value of about 180 watts. A conventional safety thermostat **394**, connected to the elements of the heating jacket, is also provided between the insulating jacket **388** and bottle **382**. The safety thermostat prevents the rinse liquid temperature from exceeding a preset value, preferably about 50° C. A thermistor temperature sensor **391** with leads **393** extends through the cap **386** into the upper zone of the bottle **382**. An liquid inlet tube **394** extends through the cap **386** to the bottom of the neck **384**, and an outlet tube **396** extends through the cap **386** to the bottom of the bottle **382**.

This unique configuration provides a highly uniform liquid output temperature. The colder water entering through the inlet tube **394**, being more dense than the heated liquid in the bottle, sinks downward past the heated container walls and is heated. The displaced liquid rises upward in the container. This stirring motion thoroughly mixes the liquid without the need for an agitator, producing a highly uniform outlet liquid temperature. Thermistor **391** constantly monitors the liquid temperature, providing a signal to the control system which is used to determine when the heating elements in jacket **390** should be energized.

FIG. **19B** illustrates an alternative embodiment of the rinse liquid container and associated heating components of the present which is similar to the structure illustrated by

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FIG. **19A** except that the inlet tube **394** of the embodiment of FIG. **19** functions as an outlet tube **394A** and outlet tube **396** of the embodiment of FIG. **19** functions as an inlet tube **396A**, i.e., the inlet and outlet lines have been reversed. This arrangement prevents the build up of air or gas in the bottle **384**. Additionally, the inlet tube **396A** has been provided with perforations **396B** for obtaining mixing as the bottle **384** is replenished with liquid.

FIG. **20A** is a bottom, isometric view of one embodiment of a reagent container support carousel **10**. According to this embodiment, the reagent container carousel **10** has feet **800**, **801** and **802** which rest in respective matching recesses in the reagent carousel support **314** (FIG. **15**) in only one position. This insures that the reagent carousel **10A** and the reagent bottle receptors **11** are always positioned in predetermined orientation on the carousel support **314**.

The feet **800**, **801** and **802** also function as supporting feet when the reagent support carousel **10** is removed. Refrigeration of the reagents is often required during their storage. The reagent container carousel **10**, with the reagent bottles supported thereon, can be lifted from the carousel support **314** and placed in a refrigerator, supported by the feet **800**, **801** and **802**.

Indexing metal homing block **803** is mounted on the reagent container carousel **10** and rotates with the carousel **10**. A conventional metal proximity detector (not shown) is mounted on the upper plate **8** at an position which places it adjacent the rotational path of the homing block. A change in electrical signal from the proximity detector indicates that the metal homing block is in the 'home' position adjacent the block.

FIG. **20B** is an alternative embodiment of a reagent support carousel **10A** and associated carousel support **314A** wherein a handle **804** has been provided to assist in the removal and replacement of the reagent support carousel **10A** as described above. In this embodiment, the carousel **10A** is provided with a plurality of feet **800A**, for example, five feet, which are substantially cylindrical elements with beveled edges **805**, and fit into corresponding and matching circular openings **802A**, formed in the associated carousel support **314A**. The feet **800A** and opening **802A** are positioned so that the carousel **10A** will fit into the support **314A** in only one position such that the carousel **10A** is always positioned in a predetermined orientation on the support **314A**. The support **314A** is provided with a central hub **806** which is received in a central opening **807** formed in the carousel **10A**, the hub being provided with beveled edges **808**. Engagement of the carousel **10A** and the support **314A** is best seen in FIG. **20C**. Except for the above described differences, the carousel **10A** and the support **314A** are the same as previously described.

FIG. **21** is a fragmentary cross-sectional view taken along the line D—D in FIG. **11**. Indexing block **229** is a metal block. Proximity sensor **610** is supported on the underside of plate **22** by bracket **611**. The proximity sensor **610** emits an electrical signal through leads **612** which changes when the metal block **229** is positioned in the 'home' position immediately above the sensor.

The homing systems of the reagent carousel **10** and slide support carousel **24** operate in a similar manner. Presence of an indexing block adjacent the sensor produces a signal indicating that the carousel is in a "home" position, and provides a reference for subsequent indexed movements of the respective stepper motor drive and subsequent indexed movements of the respective carousel.

FIG. **22** is a schematic view of the pneumatic system of the automated immunostaining apparatus of this invention.

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The air supply for the system is supplied by air compressor **80** and air filter **82**. The output line **400** from the air filter **82** is connected to the input port of air pressure regulator **86** where it is regulated to a constant output pressure of about 25 psi. Diaphragm pressure switch **402** communicates with the air pressure regulator **86** outlet line **403** through line **404**. Diaphragm pressure switch **402** closes the system circuit breaker **406** when the pressure in line **404** is at least 22 psi. Failure of the air compressor and resulting drop in line pressure automatically deactivates the system.

The air output branch line **408** lead is connected by line **410** with tipper air cylinder three way control solenoid valve **412**. When in an "open" position, solenoid valve **412** provides communication between input line and cylinder **278**. This permits pressurized air to pass from line **410** to air cylinder **278**, thus pressing tipper tip **148** (FIG. 14) against the respective slide support tab **112** and tipping the slide support **206**. When solenoid valve **412** returns to the vent position, the air cylinder **278** communicates with atmosphere, permitting the air cylinder **278** to return to its resting position. Tipper tip **148** then rises to its resting position, allowing the slide support to also return to its horizontal position. Three way solenoid valves **416** and **420** operate in an identical way, providing communication between the air inlet lines **414** and **418** and the respective air cylinders **280** and **282** when in the open position and actuating respective tipper tips **284** and **286**. They also open communication between the air cylinders **280** and **282** and the atmosphere in the vent position, allowing the tipper tips to return to their elevated position.

Branch line **422** leads from line **408** to the reagent dispenser three way control solenoid valve **424**. When energized to an "open" position, solenoid valve **424** permits pressurized air to pass from line **422** to air cylinder input line **300**, causing rod **302** and foot **306** (FIG. 15) to press the reagent dispenser bottle **12** downward, emitting a precise volume of reagent liquid. When solenoid valve **424** is in the vent position, the air cylinder **18** and the reagent bottle **12** return to their resting positions.

Branch line **426** leads from line **403** to branched lines **428** and **430**. Branch line **428** leads to pressure regulator **38**, providing an output pressure of 10 psi in output line **431**. Three way solenoid valve **432**, when in the open position, provides communication between air input line **431** to the evaporation inhibitor liquid reservoir container **434** through lines **436** and **438**. It also delivers pressurized air to the rinse liquid supply container **44** through line **440**, rinse solution reservoir **441** and supply conduit **443**. When solenoid valve is opened to atmosphere (vent position), air in line **436** and in containers **44** and **434** is bled or vented to the atmosphere. This permits removal, opening or replacement of reservoir container **434**, or opening or removal of supply container **441**. The pressured air in containers **434** and **441** forces liquid through respective output conduits **442** and **443**.

Conduit **442** leads to two way solenoid valve **446**, which has an outlet conduit **448** leading to the evaporation inhibitor application block **212** and associated nozzles. When the solenoid **446** is opened, evaporation inhibitor liquid is emitted from nozzles **294** (FIGS. 14 and 15) onto the surface of the respective slide **234**.

Conduit **444** delivers pressurized rinse liquid from heated rinse liquid container **44** to branch conduits **450**, **452** and **454** leading to conventional rinse liquid two way solenoid valves **460**, **462** and **464**. When the solenoid valves **460**, **462** and **464** are opened, pressurized rinse liquid is delivered to the respective rinse blocks **200**, **202** and **204** through supply

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conduits **260**, **268** and **276**. The pressurized rinse liquid is emitted by the rinse blocks onto the slides positioned in the respective station (FIG. 13).

Branch line **430** leads to pressure regulator **64**, providing an output pressure of 15 psi in output conduit **466** leading to vortex mixer air control two way solenoid valve **468**. When in the open position solenoid valve **468** delivers pressurized air to output conduit **470** connected thereto. Conduit **470** leads to branch lines **472** and **474** leading to vortex mixing blocks **222** and **224**. The pressurized air is emitted by nozzles **351** and **355** (FIG. 17), stirring the reagent layer on the respective slides **234**.

FIG. 23 is a schematic drawing of the 120 volt AC power distribution in the apparatus of this invention. The power circuit to power line filter **500** includes a main fuse **504** and main power switch **506**. 120 Volt AC power to the air compressor **80** is provided by line **511** from the line fuse **510** in the I/O board **508**. 120 Volt AC power to the air compressor cooling fan **514** is provided by line **513** from line fuse **512** in the I/O board **508**. 120 Volt AC power to the electronics cooling fan **518** is provided by line **517** from line fuse **516** in the I/O board **508**. 120 Volt AC power to the 24 volt DC power supply is provided by line **521** from line fuse **520** in the I/O board **508**. 120 Volt AC power to the 5 volt/12 volt DC power supply **78** is provided by line **524** from line fuse **522** in the I/O board **508**. 120 Volt AC power to the computer card rack **529** is provided by line **528** from line fuse **526** in the I/O board **508**. 120 Volt AC power to slide heater fan relay **533** is provided by line **532** from line fuse **530** in the I/O board **508**. 120 Volt AC power to the slide heater relays **537** is provided by line **536** from fuse **534** in the I/O board **508**. 120 Volt AC power to the rinse fluid heater relay **541** is provided by line **540** from fuse **538**.

FIG. 24 is a schematic drawing of the DC power distribution in the apparatus of this invention. 12 Volt DC logic power for printer **550** is provided by line **552** from the power supply **78**. Similarly, 12 volt DC power for low slide temperature controller **68** is provided by line **554**, 12 volt power for high slide temperature controller **70** is provided by line **556**, and 12 volt power for rinse fluid temperature controller **66** is provided by line **558**. 5 Volt DC laser power for the slide bar code reader **231** is provided by line **560** from the power supply **78**, and 5 volt power for the laser of reagent bar code reader **346** is provided by line **562**. 5 Volt DC power to the liquid crystal display **34** is provided by line **564**.

24 Volt DC power is provided to the upper motor controller **566** for the stepper motor **14** by line **568**. 24 Volt DC power for the lower motor controller **570** for the stepper motor **48** is provided from power supply **76** by line **572**.

The conventional card rack **529** has a separate 5 volt/12 volt power supply **576**. 5 Volt DC logic power and 12 volt DC motor power is provided to the floppy disc drive by lines **574**.

FIG. 25 is a schematic drawing of a first portion of the computer digital I/O system in the apparatus of this invention. The control system uses a series of standard optical relays, each of which are connected to close the line to ground in the power circuit for the respective component. The optical relays provide isolation.

Communication between the optical relays and the computer digital I/O board **580** is provided by lines **582**. The two way solenoid valves **460**, **462** and **464** controlling the rinse liquid flow from heated rinse supply **44** to the respective rinse blocks **200**, **202** and **204** are energized to an open position and de-energized to a closed position by output

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signals from the computer digital I/O board **580** to the optical relays **584**, **586** and **588**. The two way solenoid valve **446** controlling the flow of evaporation control liquid from container **434** to the nozzle block **212** is energized to an open position or de-energized to a closed position by output signals from board **580** to optical relay **590**.

The three way solenoid valves **412**, **416** and **420** controlling air flow to the respective tipper air cylinders **278**, **280** and **282** are energized to an open position (causing air flow) or de-energized to a closed position (venting cylinder air to the atmosphere) by output signals from computer I/O board **580** to respective optical relays **592**, **594** and **596**. The three way solenoid valve **424** controlling air flow to the micro delivery reagent dispenser control cylinder **300** is energized to an open position (causing air flow and reagent delivery) or de-energized to a closed position (venting cylinder air to the atmosphere) by output signals from computer I/O board **580** to respective optical relay **598**. The two way solenoid valve **468** controlling air flow to the vortex air mixer blocks **220**, **222** and **224** (FIG. 17) is energized to an open position (causing air flow to the mixer blocks) or de-energized to a closed position by output signals from computer I/O board **580** to respective optical relay **600**.

The sound alarm **602** is activated to produce sound by an output signal from the computer I/O board **580** to optical relay **604**. The sound alarm **602** can be activated to sound a 'beep' by keyboard key operation, by a longer 'beep' or double 'beep' at the completion of a run, and a sustained sound during a system malfunction, for example. The three way solenoid valve **432** controlling air flow to the rinse liquid and evaporation control liquid supply containers **44** and **434** (FIG. 22) is energized to an open position (causing air flow and pressurization of the supply containers) or de-energized to a closed position (venting cylinder air from the containers to the atmosphere) by output signals from computer I/O board **580** to respective optical relay **606**.

The slide heat fan **56** speed is operated by pulse width modulation, that is, power pulses from the power relay **608**. The fan **56** is energized by an output signal to the power relay **608** from optical relay **610**. The timed signal to the optical relay **610** is received from the computer I/O board **580**. The pulse width and speed of the fan **56** is adjusted in response to heating requests from the high temperature slide controller **632** to increase the volume of heating air delivered to the air distribution manifold **30**.

The slide heater system control supplies separately controlled power to each of the resistance heating elements **331** and **332**. Low temperature heating element **332** is energized by power relay **612** upon a signal from the low slide temperature controller **614**. Thermistor **347** provides temperature information to the controller **614**. During the operation of the apparatus at the lower temperatures required for the immunohistological processes, the power to the heating element **332** is turned on when operating heat is required, in response to a low temperature signal from the low temperature controller **614**. It is turned off when the operating temperature is restored. The controller **614** also detects when the slide door switch **616** is closed. If the cabinet slide door is open, energy supply to the heating element **331** and **332** is interrupted. The heating cycle is initiated by a request for heat passed to the computer I/O board **580** through line **624** to the optical relay **622**. The computer then responds with a heating power select heat signal received by controller **614** through line **620** from optical relay **618** in response to an output signal from the computer I/O board **580**. A status signal for the slide door switch is received by the computer I/O board through line **628** and optical relay **626**.

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The high temperature heating element **331** is energized by power relay **630** upon a signal from the high slide temperature controller **632**, in response to a power command signal through optical relay **634** and line **636** from the computer digital I/O board **580**. During the operation of the apparatus at the lower temperatures required for the immunohistological processes, the power to the heating element **331** is turned on only during an initial warm-up cycle. During the warm-up cycle, heat energy is requested from the I/O board **580** through line **638** and optical relay **640**.

When the apparatus is operated at the higher temperatures required for in situ hybridization, the heating elements are energized in a different control sequence by the controllers **614** and **632**. As with the low temperature operation, both heating elements **331** and **332** are energized during the warm-up cycle. However, in the high temperature operating mode, the low temperature heating element **332** is continuously energized, and energy is supplied intermittently to the heating element **331**. In the high temperature mode, therefore, the optical relay **634** receives a power command signal from the I/O output board **580** when the high temperature controller **632** signals that more heat is required. In addition to the heater controls described above, an additional thermostat is provided in the heater circuit which turns the heater off if the heater temperature reaches 160° C., for example if the fan **56** fails.

The rinse liquid heating system resistance heater **390** (FIG. 19) is energized through power relay **642** upon a signal from rinse fluid controller **644**. Thermistor **391** monitors the rinse fluid temperature, and the controller **644** provides a signal indicating whether or not further heat energy is required. A heat request signal for heating liquid is received by the computer I/O board through line **646** and optical relay **648**. The computer responds with a heat select signal from the I/O board **680** through relay **650** and line **652**.

FIG. 26 is a schematic drawing of a second portion of the computer digital I/O system in the apparatus of this invention. The computer digital I/O board **580** receives a signal indicating closure of the air pressure switch **402** (FIG. 22) through line **670** and optical relay **672**. The computer digital I/O board **580** receives a home signal from the reagent carousel metal proximity home sensor through line **676** and optical relay **674** when the metal block **803** and the reagent carousel **10** are in the home position. The computer digital I/O board **580** receives a home signal from the slide support metal proximity home sensor **610** through line **680** and optical relay **678** when the metal block **229** and the slide support carousel **24** are in the home position.

The reagent carousel stepper motor **14** is operated by reagent carousel stepper motor controller **690** in response to commands received from the computer digital I/O board **580**. Command signals for steps (motor operation) are received through line **692**, and command signals for the direction of operation are received through line **694**. The stepper motor has a high and low torque operating mode, the low torque mode being effected by switching a resistor into the control circuit. The high torque mode is used to move the motor through the number of steps required to place a selected reagent bottle in the reagent delivery station. The low torque mode is used as a brake to hold the reagent bottle carousel in a position. The low or high torque command signal is received by the reagent carousel stepper motor controller **690** through line **698** and optical relay **696**.

The slide support carousel stepper motor **48** is operated by slide support carousel stepper motor controller **700** in response to commands received from the computer digital

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I/O board **580**. Command signals for steps (motor operation) are received through line **702**, and command signals for the direction of operation are received through line **704**. This stepper motor also has a high and low torque operating mode, activated in the same way and having the same functions as the reagent carousel stepper motor operating modes. The high torque mode is used to move the motor through the number of steps required to place a selected slide in a selected treatment zone. The low or high torque command signal is received by the slide support carousel stepper motor controller **700** through line **708** and optical relay **706**. When the door switch **616** shows an open door status, the step command signals to the stepper motors **14** and **48** are prevented. If the door switch **616** is opened during a biological processing run, any incomplete stepper motor sequence is permitted to reach completion before further step command signals are blocked.

The keyboard **710** is a conventional pressure sensitive keyboard. The switches **720–726**, **730–736**, **740–746** and **750–756** are closed by manual pressure applied to the surface of an impermeable flexible plastic layer over the switches. The switches are isolated and protected under the plastic layer and are not fouled by moisture or debris from the laboratory or operator.

In operation input lines **711**, **712**, **714** and **716** are each sequentially energized for a brief period by the computer digital I/O board **580**, and the lines **718**, **728**, **738** and **740** are each sequentially polled during this brief period. If line **718** polls positive while line **716** is energized, closure of switch **720** is indicated. In a similar manner, closure of switch **722** is indicated by a positive poll of line **718** when line **714** is energized, closure of switch **724** is indicated by a positive poll of line **718** when line **712** is energized, closure of switch **726** is indicated by a positive poll of line **718** when line **711** is energized, and the like.

FIG. **27** is schematic drawing of the computer serial and floppy disk I/O system in the apparatus of this invention. The computer RS-232 I/O port **770** sends polling signal to the slide barcode reader **231** and receives signals indicating bar code information read through line **772**. Similarly, the computer RS-232 I/O port **770** sends polling signal to the reagent carousel barcode reader **346** and receives signals indicating barcode information read through line **774**. Signals to the liquid crystal display **34** are sent through line **776** from the RS-232 I/O port **770**. The computer RS-232 I/O port **770** receives an availability polling signal from the printer **550** and sends digital data to printer **550** through line **778**.

Immunohistological methods for which the apparatus of this invention are particularly suitable are described in concurrently filed, commonly assigned patent application Ser. No. 07/488,601, filed Mar. 2, 1990, now abandoned, the entire contents of which are hereby incorporated by reference. A typical immunohistological method, as carried out with the apparatus of this invention includes the following steps.

- 1) Preparing the slides, including applying a bar code to the slide indicating the immunohistological process to be used with the sample, and manually rinsing and applying evaporation inhibiting liquid to the tissue sample surface before placement in the apparatus to prevent dehydration of the sample.
- 2) Inserting a batch of slides in the apparatus, mounting each slide in a slide support.
- 3) Closing the apparatus and beginning the treatment processing. The apparatus heating system is in the

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warm-up mode until the heating air temperature reaches the desired level.

- 4) A slide is rinsed in the first rinse station (FIGS. **11–14**) in seven rinse cycles. Each cycle includes applying a 500 μ L pulse of rinse liquid followed by tipping the slide support to effect draining. This sequence can be repeated for seven rinse cycles as the slide is moved to and pauses in each of the second and third rinse stations, for a total of twenty-one rinse cycles, for example. The slide then is treated in a seven second stay in the evaporation inhibitor and reagent solution application station (FIGS. **11**, **14** and **15**). An initial quantity of 500 μ L of an evaporation inhibiting liquid such as dodecane is applied to the slide surface. Then 200 μ L of reagent solution is applied to the slide.

As each slide poises in the reagent application zone, the appropriate reagent container is moved by the reagent carousel to the reagent application station, and a metered volume of reagent is applied to the slide. In being applied to the slide, the reagent liquid is applied to the uppermost surface (the evaporation liquid layer). It then passes through the evaporation inhibiting liquid layer to the underlying aqueous layer, a procedure which would not be possible with a conventional solid glass coverslip.

- 6) The slide is then passed to each of the vortex mixing stations (FIGS. **11**, **14**, **16** and **17**). Here vortex jets stir the reagent on the slide surface under the file of evaporation inhibiting liquid. This procedure would not be possible with a conventional solid glass coverslip.
- 7) The slide is then carried by the carousel, pausing as each slide support is sequenced through the same steps, until it returns to the initial rinse station, where the cycle is repeated. The reaction between the reagent and the tissue sample continues during this period, and slides in each of the following slide supports is subjected to the same sequence of rinse, application of evaporation inhibitor, application of reagent, stirring, and incubation.
- 8) In a typical immunohistological process using a four phase process with a peroxidase enzyme antibody label, a sequence total of five different reagents are applied as the tissue sample is passed five times through the reagent application zone. In such a process, the first reagent is a hydrogen peroxide solution required to eliminate endogenous peroxidase activity in the tissue sample. The second reagent is a primary antibody which binds selectively with an specific epitope for which the sample is being tested. The third reagent is a biotin labeled secondary antibody which binds preferentially with the primary antibody remaining on the sample following the preceding incubation and rinsing. The fourth reagent is avidin labeled with an enzyme such as a peroxidase enzyme, the avidin binding with the biotin label remaining on the sample following the preceding incubation and rinsing. The fifth reagent is a substrate solution which is converted by the peroxidase enzyme to form a detectable label such as a fluorophore or chromophore at the site of any primary antibody binding with the sample.
- 9) Following the conclusion of the substrate solution treatment and incubation, the slide typically is removed from the carousel, coverslipped with a glass coverslip and examined to determine the extent of primary antibody binding with the tissue sample.

FIG. **28** illustrates an alternative embodiment of the intermediate section **4**, including the slide support carousel

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24 and the associated slide treatment stations, which dispenses with the tipper rinse method described above and employs an alternative rinsing arrangement, using stationary slide supports, as will be more fully described hereinafter. The carousel **24** is rotated, for example, in a clockwise manner, as indicated by the arrow shown in FIG. **28**, so that each slide support **26A** and associated slide **234** is positioned in the rinse zone A, evaporator inhibitor and reagent application zone B, and agitation zone C for successive treatment and incubation as previously described above.

In the embodiment depicted by FIG. **28**, an alternative embodiment of the slide support **26A** is provided which does not pivot, but rather is fixedly supported in a predetermined position on the carousel **24** by screws or the like and structured so that the associated slide **234** is held substantially horizontally as best seen in FIGS. **29A–29B**. Referring to FIGS. **29A–29B**, the slide support **26A** has a distal end **103A**, which is juxtaposed to the center of the carousel **24**, and a proximal end **104**, which is positioned adjacent to an outer circumference of the carousel **24**.

The support **26A** comprises a support plate **102A** having a raised terminal guide end platform **106**, adjacent the proximal end **104A** and a support post **107A**, adjacent the distal end **103A**. The platform **106A** and the post **107A** cooperate to support the slide **234** in a substantially horizontal position at a predetermined vertical distance with respect to raised terminal guide tabs **108A** and **109A** between which the slide **234** is positioned.

As best seen in FIG. **29B**, the tabs **108A**, **109A** are provided with a vertical length such that the upper surface of the slide **234** is positioned above the upper ends of the guide tabs **108A**, **109A** while the respective lateral edges **111A**, **113A** of the tabs **108A**, **109A** engage the lateral sides of the slide **234**, i.e., the tabs **108A** and **109A** do not extend as far as the upper surface of the slide **234** to prevent wicking-off of any liquid on the upper surface of the slide **234** by the tabs **108A** and **109A**. The lateral edges **111A**, **113A** cooperate with the a guide edge **115A** at the platform **106A** to orient the slide **234** at a predetermined position with respect to the slide support **26A**, and thus the carousel **24**, for treatment at the various treatment stations to be describe hereinafter.

A clamping arrangement, generally indicated at **118A**, positioned at the proximal end **104A**, clamps the slide **234** to the slide support **26A**. The clamping arrangement comprises a pair of supports **119A** between which a slide engaging member **120A** is pivotally supported. Spring **121A** biases the slide engaging member **120A** to firmly hold the slide **234** against the platform **106A** and post **107A**. The slide support **26A** permits easy loading and unloading of the slide **234**, firmly holds the slide **234** in place, does not interfere with the operation of the bar code reader and prevents or minimizes the wicking, i.e., surface tension, from draining liquids off the slide **234**.

An alternative embodiment of the rinsing arrangement forming the rinse zone A is employed in the embodiment depicted by FIG. **28** which replaces the rinse blocks, and arrangement thereof, used with the tipper rinse method previously described with respect to FIG. **14**. Referring to FIG. **28**, the rinse zone A employs a first rinse block **200A**, having a single wash block nozzle, as best seen in FIGS. **30A–30B**, and a second rinse block **202A**, having a dual wash block nozzle, as best seen in FIG. **31**.

The first wash block **200A** is preferably positioned at the beginning of the rinse zone A and the second wash block **202A** is preferably positioned at the end of the rinse zone A so that the first and second wash blocks are spaced from one another. The first wash block **200A** pulses streams of rinse

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liquid onto a slide upon entering the rinse zone A and due to the meniscus effect of the rinse liquid at the edges of the slide, builds up a layer of rinse liquid which remains on the slide. After a predetermined waiting period, set by the time it takes for the slide carousel to transport a slide between the first and second wash blocks **200A**, **202A**, the second wash block **202A** uses pulsed streams of rinse liquid, alternately directed at one and then the other of the longitudinal edges of the slides, to knock or sweep the previously deposited layer of rinse liquid off of the slide.

The rinsing arrangement depicted in FIG. **28** rinses or washes the upper surface of the slides with streams or jets of pulsed rinsing liquid, for example, water, so that a low volume of rinsing liquid is used to provide a high degree of rinsing. Because the rinsing of the slides is a key limit to the sensitivity of the assays as background or noise is directly related to rinsing and sensitivity is the signal to noise, ratio, the wash blocks **200A**, **202A** precede the application of the reagent and are a preferred feature of this embodiment of the invention.

Referring to FIG. **30A**, the first wash block **200A** comprises a single wash block nozzle **201A** having a plurality of nozzle outlet openings **203A**, for example **10** or so openings, which each provide a pulsed stream of rinse liquid **204A** which impacts the rinse liquid impact zone **236** of the slide **234** as previously described. Due to the meniscus effect of the rinse liquid at the longitudinal edges **234P** and lateral edge **234L** of the slide **234**, a layer of rinse liquid **213A** is built up on the slide **234** as a result of the repeated pulsing of streams of rinse liquid during the operation of the first wash block **200A**.

As best seen in FIG. **30B**, a nozzle axis **240A** of the nozzles of block **200A** forms an angle θ with the horizontal, this angle being between 15 and 35 degrees, preferably substantially 25 degrees.

FIG. **31** illustrates the second wash block **202A** which employs a dual wash block nozzle **205A** comprising a lower set of nozzle outlet openings **206A** and an upper set of nozzle outlet openings **207A** which respectively direct streams of pulsed rinse liquid towards one or the other of the longitudinal edges **234P** of the slide **234**.

As with the first wash block **200A**, the streams of pulsed rinsing liquid, from each of the lower and upper sets of nozzle outlet openings **206A** and **207A**, preferably impact the slide **234** at the rinse liquid impact zone **236** which is upstream on the slide **234** from the tissue sample (not shown) positioned thereon. This feature of the first and second wash blocks **200A** and **202A** is important due to the fragile nature of the tissue sample positioned on the slide **234**. By directing the streams of pulsed rinsing liquid at the impact zone **236** of the slide **234**, the rinse liquid is provided with laminar flow by the time the rinse liquid reaches the tissue sample. As a result, undue damage to the fragile tissue sample is prevented.

The upper set of nozzle outlet openings **207A** is constructed so that the associated streams of rinse liquid are off-set at an angle from the longitudinal center line of the slide **234** so that the pulsed streams of rinse liquid are directed toward one of the longitudinal edges **234P** of the slide **234**. The lower set of nozzle openings **206A** is constructed so that the associated streams of rinsing liquid are also off-set at an angle from the longitudinal center line of the slide **234** so that the pulsed streams of rinse liquid are directed toward the other one of the longitudinal edges **234P** of the slide **234**. As a result of this arrangement, pulsed streams of rinse liquid are alternately and repeatedly directed to one and then the other of the longitudinal edges **234P** of the slide **234** as will be more fully described hereinafter.

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Preferably, separate plumbing and valving are provided for each of the lower and upper sets of nozzle outlet openings **206A** and **207A** of the dual wash block nozzle **205A** to permit independent operation thereof. In operation, wash block **202A** directs streams of pulsed rinsing liquid, for example from the lower set of nozzle openings **206A**, toward a single longitudinal edge **234P** of the slide **234** and after completion then directs streams of pulsed rinse liquid, for example from the upper set of nozzle opening **207A**, to the other longitudinal edge **234P** of the slide **234**. This procedure is repeated and has the effect of sweeping or knocking the layer of rinse liquid **213A** off of the slide **234**.

As with the first wash block **200A**, the nozzle axis **240** (not shown) of each of the upper and lower set of nozzle openings **207A**, **206A** forms an angle θ (not shown) with the horizontal of between 15 and 35 degrees, preferably substantially 35 degrees for the upper set of openings **207A** and substantially 25 degrees for the lower set of openings **206A**.

FIG. **32** illustrates an alternative embodiment of a vortex air mixer **220A** which in this case is a single mixer. Each of the single vortex air mixers **220A** is positioned at the inner radius of the slides **234** such that an gas jet or cone **356A** of, for example, air or the like, blows outwardly adjacent one of the longitudinal lateral edges **234P** of the associated slide **234** to effect mixing in a manner similar to that described with respect to FIG. **17**. More specifically, the gas stream **356A** impacts the surface of the evaporation liquid surface layer **360** and moves the underlying reagent solution in a circular path on the tissue section.

Each vortex mixer **220A** has a nozzle channel **350A**, including a nozzle orifice **351A**, which is supplied with pressurized air via a supply channel **358A**, the nozzle channel **350A** preferably intersecting the supply channel at a lower portion thereof. Pressurized air is supplied to the supply channel **358A** from a air supply conduit **352A** (arrows indicating the flow of air to and from the mixer **220A**) connected to a pressurized air source (not shown). Each of the vortex mixers **220A** can be supplied with pressurized air via a common supply conduit **352A** which connects and supplies each of the supply channels **358A** of the plurality of mixers **220A** illustrated in FIG. **28**.

As best seen in FIG. **28**, there are, for example twelve, single vortex mixers **220A** on the inner radius of the slides **234**. The nozzle orifice **351A** of each of the mixers **220A** is preferable positioned so that the center of the gas net or cone **356A** is approximately 2 mm above the surface of the slide **234** and 4 mm in from the adjacent edge **234X** of the slide **234** as best seen in FIG. **32**.

A first mixer **220A** is preferably positioned at station **S2** adjacent the reagent drop point station **S1** and a second mixer **220A** is positioned at station **S3**, the mixers **220A** at stations **S2** and **S3** directing the stream of air **356A** to opposite longitudinal edges **234P** of an associated slide **234** so that mixing is enhanced as described below.

The exact positioning of the remaining mixers **220A** is not critical, these mixers **220A** being positioned to provide a semi-continuous mixing. Additionally, each mixer **220A** is spaced so that they alternate in blowing the right side and then the left side of the slide **234**. That is, the even mixers blow up the right side of each slide **234** passing by and the odd mixers blow up the left side or vice versa. This enhances kinetic mixing, provides uniform coverage and averages out any possible temperature differences across each of the slides **234**. These features lead to more rapid and reproducible staining than can be obtained manually.

Additionally, the intermediate section **4** of the embodiment of FIG. **28** is provided with a bar code cleaner,

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generally indicated at **233A**, for cleaning drops of liquid off of the bar codes **233** (FIG. **32**) provided for each of the slides **234** for identification purpose as previously described. It should be noted that the bar code cleaner **233A** is equally applicable to the previously described embodiment of the invention employing the tipper rinse method described above. The bar code cleaner **233A** is positioned, for example, downstream from the reagent drop point station **S1** just beyond the first vortex agitation zone **C** as best seen in FIG. **28** and upstream and adjacent to the bar code reader position (not shown).

The bar code cleaner **223A** is illustrated in detail in FIGS. **33A**–**33B** and comprises a bar code nozzle **333A** supplied with compressed air or the like via a supply channel **334A** which is connected to a compressed air source (not shown) by supply conduit **335A**. The bar code nozzle **333A** is supported above the slide carousel **24** by support **336A**, as best seen in FIG. **33B**, and affixed to the stationary support plate **22** of the intermediate section **4**. The nozzle **333A** emits a stream or cone of air **337A** which blows across the bar code **233** of an adjacent slide **234** attached to the associated slide support **26A**. The stream of air **337A** blows drops of liquid off of the bar code **233** which otherwise interfere with the reading of the bar codes by the bar code reader.

As best seen in FIG. **33A**, the nozzle axis **338A** of the bar code nozzle **333A** forms an angle of about 45 degrees with the horizontal. Additionally, the stream of air **337A** preferably strikes the bar code **233A** in the area of the side of the bar code **233A** closest to nozzle **333A**.

Since the embodiment of the intermediate section **4** described with reference to FIG. **28** does not employ the tipper rinse method, any rinse liquid remaining on the slide after operation of the second wash block **202A** is drained from the upper surface of the slides **234** by a jet drain **148A** which is illustrated schematically by FIG. **34**. The preferred position of the jet drain **148A** is at the last rinse station of the rinse zone **A** just prior to the reagent drop point station **S1** as best seen in FIG. **28**.

The jet drain **148A** directs a fluid stream **150A** of, for example air, at substantially a 45 degree angle to the longitudinal axis of an associated slide **234** and across one corner of the distal end **104A** of the associated slide **234**. The action of the fluid stream **150A** acts to blow, aspirate or siphon the buffer remaining after the rinsing performed at the rinse zone **A** as described above.

Except for the differences noted above the embodiment so described with respect to FIG. **28** is the same as the apparatus described above in connection with the tipper rinse method and is capable of operating and performing the immunohistological methods as previously described.

What is claimed is:

1. A method of dispensing reagents onto a slide, the method comprising the steps of:

- providing at least one reagent container;
 - providing at least one slide on a slide support;
 - automatically identifying the reagent container using a computer;
 - automatically determining whether reagent in the reagent container should be dispensed onto the slide; and
 - dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide,
- wherein the step of automatically determining whether reagent in the reagent container should be dispensed onto the slide includes the steps of:

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providing a bar code reader;

reading a slide bar code placed on the slide using the bar code reader thereby acquiring slide information, the slide information indicating reagents to be applied to the slide; and

sending the slide information to the computer.

2. The method of claim 1 wherein the slide bar code identifies a slide sample placed on the slide and identifies a sequence of reagents for the slide sample.

3. The method of claim 1 further comprising the steps of: determining position information for the slide; and sending the position information to the computer.

4. The method of claim 3 wherein a slide support supports the slide and wherein the step of determining position information for the slide includes homing the slide support and determining an indexed position of a motor drive for the slide.

5. A method of dispensing reagents onto a slide, the method comprising the steps of:

providing a plurality of reagent containers in a reagent support, each of the reagent containers having a reagent barcode;

providing at least one slide on a slide support, the slide having a bar code;

providing a bar code reader;

reading the bar codes on the reagent containers;

determining reagents in the reagent containers based upon the reading of the bar codes on the reagent containers;

reading the slide bar code on the at least one slide;

determining a sequence of reagents to be applied on the at least one slide based upon the reading of the slide bar code on the slide; and

dispensing the reagents in the reagent containers based upon the sequence of reagents to be applied.

6. The method of claim 5 further comprising the steps of: determining position information for the reagent containers; and

sending the position information to the computer.

7. The method of claim 6 wherein a reagent carousel supports the reagent containers and wherein the step of determining position information for the reagent containers includes homing the reagent carousel and determining an indexed position of a motor drive for the reagent containers.

8. The method of claim 5 further comprising the steps of: determining position information for the at least one slide; and

sending the position information to the computer.

9. The method of claim 8 wherein a slide carousel supports the at least one slide and wherein the step of determining position information for the at least one slide includes homing the slide carousel and determining an indexed position of a motor drive for the at least one slide.

10. The method of claim 5 further comprising the step of moving the reagent containers and the slide support relative to one another based upon the sequence of reagents to be applied on the at least one slide.

11. An automated biological staining apparatus comprising:

a slide support for holding at least one slide;

slide support drive means for moving the slide support;

a reagent tray for supporting reagent containers;

reagent drive means for moving the reagent tray;

bar code reader;

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reagent dispensing device for applying reagent onto a particular slide; and

computer in communication with the slide support drive means, the reagent drive means, bar code reader and means for dispensing reagent,

wherein the bar code reader reads reagent bar codes on the reagent containers and at least one slide bar code on the at least one slide, and

wherein the computer automatically determines whether reagent in the reagent containers should be dispensed onto the particular slide.

12. The automated biological staining apparatus of claim 11 further comprising:

homing device connected to the reagent tray and in communication with the computer wherein the homing device determines position information for the reagent containers.

13. The automated biological staining apparatus of claim 12 wherein the reagent drive means is a motor and wherein the homing device determines an indexed position of the motor for the reagent containers.

14. The automated biological staining apparatus of claim 12 wherein the reagent drive means is a motor and wherein the motor rotates the reagent tray so that the reagent bar codes on the reagent containers are read by the bar code reader.

15. The automated biological staining apparatus of claim 11 wherein the bar code reader reads the at least one slide bar code on the at least one slide and wherein the at least one slide bar code is sent to the computer for automatically determining whether reagent in the reagent containers should be dispensed onto the particular slide.

16. The automated biological staining apparatus of claim 11 further comprising:

homing device connected to the slide support and in communication with the computer wherein the homing device determines position information for the particular slide.

17. The automated biological staining apparatus of claim 11 wherein the computer controls the movement of the reagent tray and the slide support to move relative to one another to position a reagent container over the particular slide.

18. The automated biological staining apparatus of claim 12 wherein the reagent tray is a reagent carousel and wherein the reagent drive means moves the reagent carousel to place the reagent containers in a reagent delivery zone.

19. An automated biological staining apparatus comprising:

a slide support for holding at least one slide;

slide support drive means for moving the slide support;

a reagent tray for supporting reagent containers;

reagent drive means for moving the reagent tray;

means for automatically identifying the reagent containers;

means for automatically determining whether reagent in the reagent containers should be dispensed onto a particular slide; and

reagent dispensing device for applying reagent onto a particular slide.

20. The automated biological staining apparatus of claim 19 wherein the means for automatically identifying the reagent containers includes a bar code reader, wherein the bar code reader reads reagent bar codes on the reagent containers and wherein the reagent bar codes are sent to the computer for automatically identifying the reagent containers.

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21. The automated biological staining apparatus of claim 19 wherein means for automatically determining whether reagent in the reagent containers should be dispensed onto the slide includes a bar code reader, wherein the bar code reader reads slide bar codes on the slides and wherein the slide bar codes are sent to the computer for automatically determining whether reagent in the reagent containers should be dispensed onto the particular slide.

22. The automated biological staining apparatus of claim 19 further comprising:

means for determining position information for the reagent containers, the means being in communication with the computer.

23. The automated biological staining apparatus of claim 22 wherein the means for determining position information for the reagent containers includes a homing device con-

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nected to the reagent tray and in communication with the computer wherein the homing device determines position information for the reagent containers.

24. The automated biological staining apparatus of claim 19 further comprising:

means for determining position information for the at least one slide, the means being in communication with the computer.

25. The automated biological staining apparatus of claim 24 wherein the means for determining position information for the at least one slide includes a homing device connected to the slide support and in communication with the computer wherein the homing device determines position information for the at least one slide.

* * * * *

EXHIBIT

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

(Case No. 97,008-U)

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In re Application of:)	
)	
COPELAND, et. al)	
)	Group Art Unit: 1743
Serial No.: Not yet assigned)	
)	Examiner: Not yet assigned
Filed: August 5, 1997)	
)	
For: Automated Biological)	
Reaction Apparatus)	

Asst. Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Dear Sir:

IN THE SPECIFICATION

Please delete lines 3-5 at page 1 and insert the following therefor:

This is a continuation of application Serial No. 08/906,678, filed August 5, 1997, pending, which
is a continuation of application Serial No. 08/479,415, filed June 6, 1995, U.S. Patent No. 5,654,200,
which is a division of application Serial No. 352,966, filed December 9, 1994, U.S. Patent No. 5,595,707,
which is a continuation of application Serial No. 924,052, filed August 31, 1992, abandoned, which is a
continuation-in-part of application Serial No. 488,601, filed March 2, 1990, abandoned.

At page 41, line 6, change "_____, filed March 2, 1990" to "07/488,601, filed March

2, 1990, now abandoned--, such that the sentence reads "Immunohistological methods for which the
apparatus of this invention are particularly suitable are described in concurrently filed, commonly

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assigned patent application Serial No. 07/488,601, filed March 2, 1990, now abandoned
(Attorney Docket No. 193.0007), the entire contents of which are hereby incorporated by
reference."

IN THE CLAIMS:

Please cancel claim 1 without prejudice. Please add the following claims 72-113 as follows:

72. A method of dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container;

providing at least one slide on a slide support;

automatically identifying the reagent container using a computer;

automatically determining whether reagent in the reagent container should be dispensed onto
the slide; and

dispensing the reagent in the reagent container onto the slide based on the determination of
whether the reagent in the reagent container should be dispensed onto the slide.

73. The method of claim 72 wherein the step of automatically identifying the reagent
container using a computer includes the steps of:

providing a bar code reader;

reading a reagent bar code placed on the reagent container using the bar code reader thereby
acquiring reagent information; and

sending the reagent information to the computer.

74. The method of claim 73 further comprising the steps of:

determining position information for the reagent container; and

sending the position information to the computer.

75. The method of claim 74 wherein a reagent carousel supports the reagent container and wherein the step of determining position information for the reagent container includes homing the reagent carousel and determining an indexed position of a motor drive for the reagent container.

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76. The method of claim 73 wherein the reagent bar code identifies the reagent in the reagent container.

77. The method of claim 76 wherein the step of automatically identifying the reagent container using a computer is performed at a beginning of a slide treatment operation.

78. The method of claim 77 wherein the step of automatically identifying the reagent container using a computer further includes correlating a position of the reagent container with the reagent carousel.

79. The method of claim 73 wherein the reagent container is in a reagent carousel and wherein the step of automatically identifying reagent further includes the step of rotating the reagent carousel so that the reagent bar code on the reagent container is read by the bar code reader.

80. The method of claim 72 wherein the step of automatically determining whether reagent in the reagent container should be dispensed onto the slide includes the steps of:

providing a bar code reader;

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reading a slide bar code placed on the slide using the bar code reader thereby acquiring slide information, the slide information indicating reagents to be applied to the slide; and
sending the slide information to the computer.

81. ~~The method of claim 80 wherein the slide bar code identifies a slide sample placed on the slide and identifies a histochemical process for the slide sample.~~

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82. ~~The method of claim 80 further comprising the steps of:~~
determining position information for the slide; and
sending the position information to the computer.

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83. ~~The method of claim 82 wherein a slide support supports the slide and wherein the step of determining position information for the slide includes homing the slide support and determining an indexed position of a motor drive for the slide.~~

84. ~~The method of claim 72 further comprising the step of moving the reagent container and the slide support relative to one another to position the reagent container over the slide.~~

85. ~~The method of claim 84 wherein a reagent carousel supports the reagent container and wherein the step of moving the reagent container and the slide support relative to one another includes moving a drive plate which supports the reagent carousel to place the reagent container in a reagent delivery zone.~~

86. The method of claim 72 wherein the step of dispensing the reagent in the reagent container onto the slide includes the step of pressuring the reagent container thereby metering a volume of reagent onto the slide.

87. The method of claim 86 wherein the step of pressuring the reagent container includes activating an air cylinder to move downward into positive contact with the reagent container.

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88. A method of dispensing reagents onto a slide, the method comprising the steps of:
providing a plurality of reagent containers in a reagent support, each of the reagent containers having a reagent barcode;
providing slides on a slide support;
providing a bar code reader;
reading the bar codes on the reagent containers;
determining reagents in the reagent containers based upon the reading of the bar codes on the reagent containers;
reading the bar code on the slides;
determining a sequence of reagents to be applied on the slides based upon the reading of the bar code on the slides; and
dispensing the reagents in the reagent containers based upon the sequence of reagents to be applied.

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89. The method of claim 88 further comprising the steps of:
determining position information for the reagent containers; and

sending the position information to the computer.

120. The method of claim ⁶⁰~~89~~ wherein a reagent carousel supports the reagent containers and wherein the step of determining position information for the reagent containers includes homing the reagent carousel and determining an indexed position of a motor drive for the reagent containers.

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91. The method of claim 88 further comprising the steps of:
determining position information for the slides; and
sending the position information to the computer.

92. The method of claim 91 wherein a slide carousel supports the slides and wherein the step of determining position information for the slides includes homing the slide carousel and determining an indexed position of a motor drive for the slides.

93. The method of claim 88 further comprising the step of moving the reagent containers and the slide support relative to one another based upon the sequence of reagents to be applied on the slide.

94. A method of dispensing reagents onto a slide, the method comprising the steps of:
providing at least one reagent container;
providing at least one slide on a slide support;
automatically identifying the reagent container using a computer;

moving the reagent container and the slide support relative to one another to position the reagent container over the slide; and

dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide.

95. The method of claim 94 wherein the step of automatically identifying the reagent container using a computer includes the steps of:

providing a bar code reader;

reading a reagent bar code placed on the reagent container using the bar code reader; and

sending information from the reading of the reagent bar code to the computer.

96. The method of claim 95 wherein the reagent container is supported on a reagent carousel and wherein the step of moving the reagent container and the slide support relative to one another includes moving a drive plate which supports the reagent carousel to place the reagent container in a reagent delivery zone.

97. The method of claim 94 wherein the step of dispensing the reagent in the reagent container onto the slide includes the steps of:

pushing downward on the reagent container; and

applying a metered volume of reagent onto the slide.

98. The method of claim 97 wherein the step of pushing downward on the reagent container includes activating an air cylinder to move downward in order to push the reagent container.

99. An automated biological staining apparatus comprising:

a slide support for holding slides;

slide support drive means for moving the slide support;

a reagent tray for supporting reagent containers;

reagent drive means for moving the reagent tray;

bar code reader; and

computer in communication with the slide support drive means, the reagent drive means, and

bar code reader,

wherein the bar code reader reads reagent bar codes on the reagent containers and wherein the reagent bar codes are sent to the computer for automatically identifying the reagent containers.

¹² 100. The automated biological staining apparatus of claim ¹¹ 99 further comprising:

homing device connected to the reagent tray and in communication with the computer wherein the homing device determines position information for the reagent containers.

¹³ 101. The automated biological staining apparatus of claim ¹² 100 wherein the reagent drive means is a motor and wherein the homing device determines an indexed position of the motor for the reagent containers.

¹⁴ 102. The automated biological staining apparatus of claim ^{12, 13} 100 wherein the reagent drive means is a motor and wherein the motor rotates the reagent tray so that the reagent bar codes on the reagent containers are read by the bar code reader.

103. The automated biological staining apparatus of claim 99 wherein the bar code reader reads slide bar codes on the slides and wherein the slide bar codes are sent to the computer for automatically determining whether reagent in the reagent containers should be dispensed onto the slides.

104. The automated biological staining apparatus of claim 99 further comprising:
homing device connected to the slide support and in communication with the computer
wherein the homing device determines position information for the slides.

105. The automated biological staining apparatus of claim 99 wherein the computer moves the reagent tray and the slide support relative to one another to position the reagent containers over the slides.

106. The automated biological staining apparatus of claim 105 wherein the reagent tray is a reagent carousel and wherein the reagent drive means moves the reagent carousel to place the reagent containers in a reagent delivery zone.

107. An automated biological staining apparatus comprising:
a slide support for holding slides;
slide support drive means for moving the slide support;
a reagent tray for supporting reagent containers;
reagent drive means for moving the reagent tray;

means for automatically identifying the reagent containers;

means for automatically determining whether reagent in the reagent containers should be dispensed onto the slide; and

computer in communication with the slide support drive means, the reagent drive means, the

means for automatically identifying the reagent containers, and the means for automatically determining whether reagent in the reagent containers should be dispensed onto the slide.

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108. The automated biological staining apparatus of claim 107 wherein the means for

automatically identifying the reagent containers includes a bar code reader, wherein the bar code reader

reads reagent bar codes on the reagent containers and wherein the reagent bar codes are sent to the

computer for automatically identifying the reagent containers.

109. The automated biological staining apparatus of claim 107 wherein means for

automatically determining whether reagent in the reagent containers should be dispensed onto the slide

includes a bar code reader, wherein the bar code reader reads slide bar codes on the slides and wherein

the slide bar codes are sent to the computer for automatically determining whether reagent in the reagent

containers should be dispensed onto the slides.

110. The automated biological staining apparatus of claim 107 further comprising:

means for determining position information for the reagent containers, the means in communication with the computer.

23 111. The automated biological staining apparatus of claim 110 wherein the means for determining position information for the reagent containers includes a homing device connected to the reagent tray and in communication with the computer wherein the homing device determines position information for the reagent containers.

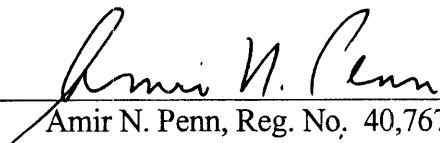
A 2
Sub 7
E 11
112. The automated biological staining apparatus of claim 107 further comprising:
means for determining position information for the slides, the means in communication with the computer.

Sub 7
E 12
113. The automated biological staining apparatus of claim 112 wherein the means for determining position information for the slides includes a homing device connected to the slide support and in communication with the computer wherein the homing device determines position information for the slides.

Respectfully submitted,

McDonnell Boehnen Hulbert & Berghoff

By:


Amir N. Penn, Reg. No. 40,767
Attorney for Applicant

DATED:

EXHIBIT

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HA



**UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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EXAMINER

REF. P	
ART UNIT	PAPER NUMBER

1743
DATE MAILED: 05/10/01

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No.		Applicant(s)		
	09/452,309		COPELAND ET AL.		
	Examiner		Art Unit		
	P. K. Bex		1743		

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on 26 April 2001.

2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) 72-113 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☐ Claim(s) _____ is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some * c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. _____.

3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) 16) <input checked="" type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) 17) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____	18) <input type="checkbox"/> Interview Summary (PTO-413) Paper No(s). _____ 19) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) 20) <input type="checkbox"/> Other: _____
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Application/Control Number: 09/452,309

Page 2

Art Unit: 1743

DETAILED ACTION

1. The cancellation of claims 1-71 and the addition of claims 72-113 is acknowledged and has been entered into the record.

Election/Restrictions

2. The restriction requirement filed February 26, 2001 is withdrawn in response to the personal interview with Amir Penn on April 26, 2001.

Claim Rejections - 35 USC § 112

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 81, 88-93, 99-113 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 81, line 2, provides for the use of slide bar code for a histochemical process, but, since the claim does not set forth any steps involved in the method/process, it is unclear what method/process applicant is intending to encompass. A claim is indefinite where it merely recites a use without any active, positive steps delimiting how this use is actually practiced. It is indefinite as to what is meant by the limitation "histochemical process".

Claim 88, line 9, recites the step of "reading the bar code on the slide ". There is insufficient antecedent basis for this limitation in the claim. No bar code on a slide is disclosed.

Claim 99, line 2, "a slide support *for holding slides*" does not provide a positive recitation for the slides. It is suggested that a limitation which discloses "a plurality of slides" be added into the claim. This same reasoning applies to claim 107.

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Art Unit: 1743

Page 3

Claim 103, line 2, "the slides" lacks proper antecedent basis. Same deficiencies were found in claims 103-105 and 109.

Claim 105, line 1, "the computer moves" is unclear and confusing. How does the computer *move* the reagent tray? For examination purposes, this has been interpreted to mean a computer which "controls" the reagent rotor.

Claim 107, lines 7-10, recite "means for automatically determining whether reagent in the reagent containers should be dispensed" and "computer in communication with ...the means for automatically determining whether reagent in the reagent containers should be dispensed". It is not clear as to how these are two different limitations. Examiner believes that the computer performs the step that automatically determines whether reagent in the reagent containers should be dispensed. Clarification is requested.

Claim Rejections - 35 USC § 102

5. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

6. Claims 72, 84-87, 94, 97-98, 107, 110, 112 are rejected under 35 U.S.C. 102(b) as being anticipated by Bogen *et al* (USP 5,645,114).

Bogen *et al* an automatic method and apparatus for immunocytochemistry substantially as claimed. The apparatus comprises a rotatable slide support carousel 504 for mounting a plurality of slide supports 532 comprising samples, a drive means for rotating the slide support carousel (column 5, line 52). Additionally, Bogen *et al* teach a reagent rotor 504 positioned

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Page 4

Art Unit: 1743

above the slide rotor and a plurality of reagent containers. The reagent containers for applying a predetermined quantity of reagent onto the slide which is based on a program containing histochemical protocols regarding the particular reagent to be used on the sample. Further, Bogen *et al* disclose the use of a program which supplies the microprocessor with information regarding the location of the reagents on the reagent rotor and the location of slides on the slide rotor (column 8, lines 1-26, Figs 5-11B).

Claim Rejections - 35 USC § 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

8. The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

9. Claims 72-83, 86-93, 99-104, 107-113 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stark *et al* (*J. Immunol. Methods* 107:89-92 (1988)) in view of Sakurada (USP 4,346,056) or Saralegui *et al* (USP 5,439,645).

Stark *et al* disclose an automatic method and apparatus for immunocytochemistry. The apparatus comprises a rotatable slide support carousel for mounting a plurality of slide supports

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Page 5

Art Unit: 1743

7, drive means 4 for rotating the slide support carousel, and reagent containers 3 for applying a predetermined quantity of reagent into a slide (Fig. 1). Stark *et al* fail to specifically recite sensors for detecting positions of slide samples or reagent containers, or a bar code reader for identifying types of reagents.

Sakurada disclose the rotation position of the reagents as well as, the different types of reagents used, which are detected and identified by sensors (column 3, lines 20-31, column 4 line 3- column 6, line 7, Figs. 3, 5). Similarly, Saralegui *et al* teach a plurality of sample containers on a carousel, the sample containers comprising bar code labels for identifying the appropriate process for the sample. Moreover, Saralegui *et al* teaches the use electro-mechanical sensors, e.g. home sensors 106 to detect when the carousel is at the "home" position and subsequently determining the indexed position of the stepper motor drive 88 for the containers from this "home" position (column 8, line 45- column 9, line 3, Fig. 3, 5).

Such a delivery control mechanism is considered well known for its reliable functions, speed of operations in the autoanalyzer art. Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have provided the modified apparatus of Stark *et al* with sensors for detection and identification of containers and its movements, as taught by Sakurada or Saralegui *et al*, in order to carry out the sample analysis automatically, reliably and simply (see Summary of Invention of Saralegui *et al*).

10. Claims 84, 94-98, 105-106 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stark *et al* (*J. Immunol. Methods* 107:89-92 (1988)) in view of Sakurada (USP 4,346,056) or Saralegui *et al* (USP 5,439,645) as applied to claim 72 above, and further in view of Rokugawa (USP 4,844,868).

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Art Unit: 1743

Page 6

Stark *et al*, Sakurada and Saralegui *et al* as previously discussed, fail to specifically recite the step of moving the reagent container and slide support relative to one another to position the reagent container over the slide. Rokugawa teaches a method and apparatus for delivering reagents to reaction containers wherein a plurality of reagents 68 are supported on a reagent carousel 64 which positioned over a reaction carousel (Fig. 1). Such an arrangement of reagent delivery system would provide an improved automatic chemical analysis with which it is possible to eliminate dead space in the reagent passage, reduce size, reduce distribution time and eliminate cross contamination (column 1, lines 38-43). Note: the reagent carousels of Rokugawa would inherently include homing and indexing means in order to identify each position of reagent containers and rotate an appropriate reagent container to the reagent dispensing zone which is positioned under the plunger mechanism 100, since different reagents are used in the Rokugawa system.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have provided the apparatus of Stark *et al*, Sakurada and Saralegui *et al* with a reagent distributor as taught by Rokugawa, in order to provide an improve automatic chemical analysis with which it is possible to eliminate dead space in the reagent passage, reduce size, reduce distribution time and eliminate cross contamination.

Conclusion

11. No claims allowed.
12. The prior art made of record and not relied upon is considered pertinent to applicant's disclose are Sasaki *et al*, Bogen *et al*, Copeland *et al*, Tseung *et al* Healey *et al*, Karwzak *et al*,

Application/Control Number: 09/452,309

Page 7

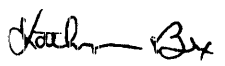
Art Unit: 1743

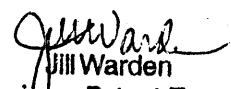
Stokes *et al*, Kerr *et al*, Bowman *et al* and Andersen *et al*. They are cited of interest in that they show various methods and apparatus for dispensing reagents into a slide or container.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to P. Kathryn Bex whose telephone number is (703) 306-5697.

The fax number for the organization where this application or proceeding is assigned is (703) 305-7718 for official papers prior to mailing of a Final Office Action. For official papers after mailing of a Final Office Action, use fax number (703) 305-3599. For unofficial or draft papers use fax number (703) 305-7719. Please label all faxes as official or unofficial. The above fax numbers will allow the paper to be forwarded to the examiner in a timely manner.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0661.


P. Kathryn Bex
Patent Examiner
AU 1743
05/04/01


Jill Warden
Supervisory Patent Examiner
Technology Center 1700

EXHIBIT

4



R. Haworth
#10
5:23:01

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 97,008-U)

In re Application of:)
)
Copeland et al.)
)
Serial No.: 09/452,309)
)
Filed : December 1, 1999)
)
For: AUTOMATED BIOLOGICAL)
REACTION APPARATUS)

Group Art Unit: 1743

Examiner: Bex

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TC 1700

Commissioner for Patents
Washington, D.C. 20231-9999

INFORMATION DISCLOSURE STATEMENT

Dear Sir:

This prior art statement is filed under 37 C.F.R. §§1.97-1.98 in compliance with the duty of disclosure set forth in 37 C.F.R. §1.56. Applicants respectfully request that this statement together with the attached Form PTO-1449 and accompany references be placed in the file for the subject application.

In the judgment of the undersigned, the references listed on the attached Form PTO-1449 may be material to the Examiner's consideration of the presently pending claims. However, the references have not been reviewed in sufficient detail to make any other representation and, in particular, no representation is intended as to the relative relevance between references, whether cited in this statement or prior statements. This statement is not a representation that the listed references have effective dates early enough to be "prior art" within the meaning of 35 U.S.C. §102.

In accordance with MPEP Sections 609 and 707.05(b), it is requested the documents

cited (including any cited in applicant's specification which are not repeated on the attached Form PTO-1449) be given thorough consideration and that they be cited of record in the prosecution history of the present application by initialing on Form PTO-1449. Such initialing is requested even if the Examiner does not consider a cited document to be sufficiently pertinent to use in a rejection, or otherwise does not consider it to be prior art for any reason, or even if the Examiner does not believe that the guidelines for citation have been fully complied with. This is requested so that each document becomes listed on the face of the patent issuing on the present application.

CITED REFERENCES

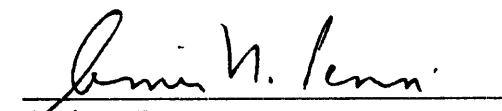
U.S. Patents

1. U.S. Patent No. 4,678,752, published July 7, 1987.
2. U.S. Patent No. 4,729,661, published March 8, 1988.
3. U.S. Patent No. 4,855,109, published August 8, 1989.
4. U.S. Patent No. 5,051,238, published September 24, 1991.
5. U.S. Patent No. 5,229,074, published July 20, 1993.
6. U.S. Patent No. 5,646,046, published July 8, 1997.
7. U.S. Patent No. 5,656,493, published August 12, 1997.

Respectfully submitted,

McDonnell Boehnen Hulbert & Berghoff

Dated: May 10, 2001


Amir N. Penn
Registration No. 40,767

FORM PTO-1449
(Rev. 2-32)

OTPE JC183
MAY 14 2001
PATENT & TRADEMARK OFFICE

U.S. Department of Commerce
Patent and Trademark Office

INFORMATION DISCLOSURE
STATEMENT BY APPLICANT
(Use several sheets if necessary)

Atty. Docket No.
97,008-U

Serial No.
09/452,309

Applicant:
Copeland et al.

Filing Date:
August 5, 1997

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TC 1700
Group:
1743

U.S. PATENT DOCUMENTS

Examiner Initial		Document Number	Date	Name	Class	Subclass	Filing Date if Appropriate
<div>BS</div> <div>↓</div> <div>BS</div>	1	U.S. Patent No. 4,678,752	07/07/87	Thorne et al.	<div></div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div>	<div></div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div>	
	2	U.S. Patent No. 4,729,661	03/08/88	Bell			
	3	U.S. Patent No. 4,855,109	08/08/89	Muraishi et al.			
	4	U.S. Patent No. 5,051,238	09/24/91	Umetsu et al.			
	5	U.S. Patent No. 5,229,074	07/20/93	Heath et al.			
	6	U.S. Patent No. 5,646,046	07/08/97	Fischer et al.			
	7	U.S. Patent No. 5,656,493	08/12/97	Mullis et al.			

FOREIGN PATENT DOCUMENTS

Document Number								Date	Country	Class	Subclass	Translation	
												Yes	No

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc).

EXAMINER

DATE CONSIDERED

Kalhuyn Box

7/6/01

EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication.

EXHIBIT

5

D. Lawrence
#146
6.29.01
 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
 (Case No. 97,008-U)

In re Application of:)	
)	
COPELAND, et. al)	
)	Group Art Unit: 1743
Serial No.: 09/452,309 ✓)	
)	Examiner: Bex, P.
Filed: August 5, 1997 ✓)	
)	
For: Automated Biological)	
Reaction Apparatus)	

Commissioner for Patents
 Washington, DC 20231

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 JUN 27 2001
 TECHNOLOGY CENTER 1700

AMENDMENT

Dear Sir:

Applicants hereby respond to the Office Action dated May 10, 2001.

IN THE CLAIMS:

Please amend claims ~~81~~, ~~88~~, ~~94~~, ~~99~~, ~~105~~ and ~~107~~ as follows. A marked up version of the amended claims, to show all the changes, is attached hereto on pages separate from the amendment in accordance with 37 CFR 1.121(c)(1)(ii).

2 ~~81~~. (Amended) The method of claim ~~80~~ wherein the slide bar code identifies a slide sample placed on the slide and identifies a sequence of reagents for the slide sample.

88 88. (Amended) A method of dispensing reagents onto a slide, the method comprising the steps of:

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providing a plurality of reagent containers in a reagent support, each of the reagent containers having a reagent barcode;

providing slides on a slide support, the slides having at least one slide bar code;

providing a bar code reader;

reading the bar codes on the reagent containers;

determining reagents in the reagent containers based upon the reading of the bar codes on the reagent containers;

reading the slide bar codes on the slides;

determining a sequence of reagents to be applied on the slides based upon the reading of the slide bar codes on the slides; and

dispensing the reagents in the reagent containers based upon the sequence of reagents to be applied.

94. (Amended) A method of dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container;

providing at least one slide on a slide support;

automatically identifying the reagent container using a computer;

automatically determining whether the reagent in the reagent container should be dispensed onto the slide;

moving the reagent container and the slide support relative to one another to position the reagent container over the slide; and

dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide.

99. (Amended) An automated biological staining apparatus comprising:

a slide support for holding slides;

slide support drive means for moving the slide support;

a reagent tray for supporting reagent containers;

reagent drive means for moving the reagent tray;

bar code reader;

reagent dispensing device for applying reagent onto a particular slide; and

computer in communication with the slide support drive means, the reagent drive means, bar code reader and means for dispensing reagent,

wherein the bar code reader reads reagent bar codes on the reagent containers and slide bar codes on the slides, and

wherein the computer automatically determines whether reagent in the reagent containers should be dispensed onto the particular slide.

103. (Amended) The automated biological staining apparatus of claim 99 wherein the bar

code reader reads slide bar codes on the slides and wherein the slide bar codes are sent to the

computer for automatically determining whether reagent in the reagent containers should be dispensed onto the particular slide.

114. (Amended) The automated biological staining apparatus of claim 99 further comprising:

homing device connected to the slide support and in communication with the computer wherein the homing device determines position information for the particular slide.

105. (Amended) The automated biological staining apparatus of claim 99 wherein the computer controls the movement of the reagent tray and the slide support to move relative to one another to position the reagent containers over the particular slide.

107. (Amended) An automated biological staining apparatus comprising:

- a slide support for holding slides;
- slide support drive means for moving the slide support;
- a reagent tray for supporting reagent containers;
- reagent drive means for moving the reagent tray;
- means for automatically identifying the reagent containers;
- means for automatically determining whether reagent in the reagent containers should be dispensed onto a particular slide; and
- reagent dispensing device for applying reagent onto a particular slide.

109. (Amended) The automated biological staining apparatus of claim 107 wherein means for automatically determining whether reagent in the reagent containers should be dispensed onto the slide includes a bar code reader, wherein the bar code reader reads slide bar codes on the slides and wherein the

slide bar codes are sent to the computer for automatically determining whether reagent in the reagent containers should be dispensed onto the particular slide

REMARKS

Applicants wish to thank Examiner Bex and Supervisory Patent Examiner Warden for the personal interview conducted on June 21, 2001. Claims 72-113 are in the application for consideration.

Claim Rejections – 35 U.S.C. §112

In Paragraph 4 of the Office Action, claims 81, 89-93 and 99-113 were rejected under 35 U.S.C. §112, second paragraph “as being indefinite for failing to point out and distinctly claim the subject matter which applicants regard as the invention.” Specifically, the terms “histochemical process” (claim 81), “reading the bar code on the slide” (claim 88), “a slide support for holding slides” (claims 99 and 107), “the slides” (claims 103-105 and 109), “the computer moves” (claim 105), “means for automatically determining whether reagent in the reagent containers” (claim 107), and “computer in communication with . . . the means for automatically determining whether reagent in the reagent containers should be dispensed.” Applicants have amended claims 81, 88, 99, 103-105, 107 and 109 where it is believed appropriate.

Claim Rejections – 35 U.S.C. §102

In Paragraph 6 of the Office Action, claims 72, 84-87, 94, 97-98, 107, 110 and 112 were rejected under 35 U.S.C. §102(b) as being anticipated by Bogen et al. (U.S. Patent No. 5,645,114).

The Examiner asserted that the Bogen patent discloses an automated method and apparatus for immunocytochemistry comprising a rotatable slide support carousel 504 for mounting a plurality of slide

supports 532 comprising samples, a drive means for rotating the slide support carousel, a reagent rotor 504 positioned above the slide rotor and a plurality of reagent containers. The Examiner further asserts that the reagent containers for applying a predetermined quantity of reagent onto the slide is based on programming containing histochemical protocols regarding the particular reagent to be used on the sample. As support, the Examiner states that Bogen discloses the use of a program which supplies the microprocessor with information regarding the location of the reagents on the reagent rotor and the location of slides on the slide rotor.

As an initial matter, the Bogen '114 patent is not prior art to the present application. The Bogen '114 patent was filed on May 31, 1994 as Application Serial No. 08/251,597 and is a continuation-in-part of Application Serial No. 07/881,397 filed on May 11, 1992 (now U.S. Patent No. 5,316,452). By contrast, the present invention claims priority to Application Serial No. 07/488,601 filed on March 2, 1990. A copy of Application Serial No. 07/488,601 is enclosed. Therefore, the Bogen '114 patent is not prior art to the present invention.

Some examples, though not exhaustive, of the support found in Application Serial No. 07/488,601 are as follows:

The apparatus preferably has bar code readers positioned to read bar codes on the sample containers or slides and on the reagent containers. (page 5, lines 17-19).

The automated immunostaining apparatus of this invention performs all steps of immunohistochemical and *in situ* DNA assays irrespective of complexity or their order, at the time and temperature, and in the environment needed. Specially prepared slides containing a bar code identifier and a mounted tissue section are placed in special support on a carousel, subjected to a preprogrammed sequence of reactions, and are removed from the carousel, ready for examination. (page 10, lines 8-17).

Bar code reader 231 above slide 205 reads a slide bar code 233 (Figs. 13 and 17) on each slide. The slide bar codes identifies the slide sample and the particular immunohistochemical process required for that sample. This information is fed into the computer and correlated with the indexed position of that slide with respect to "home", to control the sequence of reagent chemicals to be applied to that slide in the reagent application zone. (page 17, lines 13-20).

Bar code reader 346 can be mounted on post 302, positioned to scan a bar code 348 on the reagent container 12. Bar code 348 identifies the contents of the reagent bottle. At the beginning of a slide treatment operation, the reagent carousel 10 is rotated by the bar code reader 346, and the bar code on each reagent bottle is scanned. The scanned information is fed to the computer and correlated with the indexed position of the reagent carousel 10. This information is used to rotate the reagent carousel 10 to place the correct reagent bottle in the application zone for each slide treatment step for each slide. (page 23, lines 3-14).

The computer RS-232 I/O port 770 sends polling signal to the slide barcode reader 231 and receives signals indicating bar code information read through line 772. Similarly, the computer RS-232 I/O port 770 sends polling signal to the reagent carousel barcode reader 346 and receives signals indicating barcode information read through line 774. (page 37, lines 7-13).

Preparing the slides, including applying a bar code to the slide indicating the immunohistochemical process to be used with the sample, and manually rinsing and applying evaporation inhibiting liquid to the tissue sample surface before placement in the apparatus to prevent dehydration of the sample. (page 37, lines 27-32).

See also Figures 13, 14, 15, 16 and 17.

Moreover, Applicants do not believe that the Bogen patent anticipates any of the claims of the present invention. Bogen discloses only that “[a] microprocessor, not shown, controls the entire dispensing assembly 500.” A minimal discussion of the microprocessor is disclosed wherein it is provided that “an operator programs the microprocessor with the information such as the location of the reagents on the reagent rotor and the location of slides on the slide rotor. The operator then programs the particular histochemical protocol to be performed on the tissue samples.” (col. 8, lines 1-15). No further explanation of the microprocessor, e.g. its type, how it is interconnected, or how it specifically operates, is provided.

Bogen does not teach or even suggest **automatically** identifying the reagent container using a computer or **automatically** determining whether reagent in the reagent container should be dispensed onto the slide. Instead, Bogen teaches a system which relies, at least in part, on data entry from the operator. Specifically, in Bogen the operator “programs the microprocessor with the information such as the locations of reagents” (col. 8, lines 2-4) or “programs the particular histochemical protocol to be performed on the tissue samples.” (col. 8, lines 5-6). For example, the operator programs that for

reagent container position 1, reagent "A" is selected. Likewise, for slide position 1, histochemical protocol "z" is selected. The operator must then load reagent container position 1 with reagent "A" and slide position 1 with a slide requiring histochemical protocol "z". Otherwise, the system will work incorrectly. In fact, the Bogen system does not teach **any** steps of **automatic** identification or **automatic** determination. Rather, the computer runs its program under the assumption that the operator placed the reagent containers and the slides in their pre-programmed positions (*i.e.*, the operator has entered "data" in the form of placing the reagent containers and slides in the proper positions) and does not bother to check if, in fact, the reagents or slides are in their proper positions.

By contrast, the present invention automatically identifies the reagent container using a computer and automatically determines whether reagent in the reagent container should be dispensed onto the slide. The present invention does not rely on any form of data entry from the operator (*e.g.*, the placement of the reagent containers or slides in the pre-assigned positions). Thus, the claimed invention is not anticipated by the Bogen reference.

Rejection based on Stark, Sakurada and Saralegui references

In Paragraph 9 of the Office Action, claims 72-83, 86-93, 99-104 and 107-113 were rejected under 35 U.S.C. §103 as being unpatentable over Stark et al. (J. Immunol. Methods 107:89-92 (1988)) in view of Sakurada (U.S. Patent No. 4,346,056) or Saralegui et al. (U.S. Patent No. 5,439,645).

The Examiner asserted that the Stark reference discloses an automated method and apparatus for immunocytochemistry, the apparatus comprising a rotatable slide support carousel, drive means, and reagent containers. The Examiner further stated that the Stark reference fails to specifically recite sensors for detecting positions of slide samples or reagent containers or a bar code reader for identifying types of reagents.

With respect to Sakurada, which the Examiner combined with Stark in asserting that Applicants' claimed invention is obvious, the Examiner asserted that Sakurada discloses the rotation position of the reagents and the different types of reagents used, which are detected and identified by sensors. With respect to Saralegui, the Examiner asserted that Saralegui et al. teaches a plurality of sample containers on a carousel, the sample containers comprising bar code labels for identifying the appropriate process for the sample and teaches the use of electro-mechanical sensors to detect when the carousel is at the home position.

Applicants respectfully disagree with the Examiner and submit that neither the combination of Stark and Sakurada nor the combination of Stark and Saralegui render the Applicants' claimed invention obvious. Neither combination discloses or suggests all of the elements of Applicants' invention as disclosed and claimed.

The Stark reference, as discussed in the background section of the current application, describes a microprocessor controlled system including a revolving table or carousel supporting radially positioned slides. A stepper motor rotates the table, placing each slide under one of the stationary syringes positioned above the slides. The microprocessor is programmed prior to the beginning of the staining procedure, as disclosed in the following excerpts from the Stark reference:

The software to control the device was written in Assembler and the dialogue with the operator was via a standard terminal. At the start of the program the actual processing sequence could be programmed individually or, alternatively, this part of program could be deleted and a previously stored procedure requested. **For each specimen the number of slides and the names of the primary antisera were entered. The slides were inserted one after the other and the table advanced one position by actuating a pedal switch.** When all of the slides were inserted, the program requested that the pipetting units with the syringes be filled with the appropriate antibody solutions. Then the application of the primary antibody was requested. **In order to avoid errors the name of the antibody was indicated on the screen and all slides designated to receive this antibody were successively moved to the window in the lid of the device.**

* * *

The expenditure of work was considerably reduced and manual work was only necessary at the start and before the end of the staining procedure.

Page 91, columns 1-2 (emphasis added). The Stark reference teaches that the specific positions in the carousel are pre-programmed with the processing sequence. Prior to the beginning of a staining procedure, the Stark reference thus requires the entry of the slides in specific positions to match the pre-programmed sequence assigned for each of the specific positions.

Stark does not teach or even suggest **automatically** identifying the reagent container using a computer. Stark merely teaches **manual** identification of the reagent containers. Specifically, Stark teaches that the pipetting units be manually filled in predetermined syringes with antibody solutions necessary for the staining process. During a staining run, the computer in Stark does not “identify” the reagent container. Rather than automatically identifying, the programming in Stark assumes that the proper reagent was manually inserted. Moreover, Stark does not teach or even suggest **automatically** determining whether reagent in the reagent container should be dispensed onto the slide. Rather, Stark only teaches that the slides must be **manually** inserted in the proper positions based the preprogrammed sequence assigned to a particular slide position. Again, the programming in Stark does not automatically determine whether to apply reagent. Rather, the programming assumes that the proper slide was manually inserted into the proper position.

The Sakurada reference teaches the use of absolute positioning for identifying reagent vessels. The Sakurada reference requires that each time a position must be sensed, a light must be shown through the holes, as shown in Figures 7a-c. Sakurada does not disclose, or even suggest, automatically identifying the reagent container, based at least in part on information from the reagent container, using a computer. Rather, Sakurada identifies the reagent container based on its absolute position, not on any information on the reagent container. Moreover, Sakurada does not disclose, or even suggest, automatically determining, based at least in part on information from the slide, whether reagent in the

reagent container should be dispensed onto the slide. The Saralegui reference has a filing date of January 25, 1993. As discussed above, the current invention claims a filing date of March 2, 1990. Therefore, the Saralegui reference is not prior art to the present invention.

In contrast to Stark et al., Sakurada and Saralegui et al. taken alone or in any combination, Applicants disclose and claim in independent claims 72 and 94 a “method of dispensing reagents onto a slide” comprising “automatically identifying the reagent container using a computer” and “automatically determining whether reagent in the reagent container should be dispensed onto the slide”. Moreover, claim 88 recites a “method of dispensing reagents onto a slide” comprising “determining reagents in the reagent containers based at least in part upon the reagent barcodes read on the reagent containers” and “determining a sequence of reagents to be applied on the slides based at least in part upon the slide barcodes read on the slides”. These limitations describe an automated system which requires no “data entry” from the operator. The operator, therefore, is not required to place the reagent containers or slides in pre-assigned positions. Instead, the operator need only place the reagent containers on any position in the reagent support. Likewise, the operator need only place the slide in any position on the slide support. This automation is advantageous for at least three reasons: (1) increasing the reliability of the system; (2) reducing the complexity of the operation of the system; and (3) increasing the ability to process more tissue samples without reprogramming.

Reliability of the system

Reliability, without question, is critical in histological staining procedures. Errors often occur when (i) the wrong staining sequence is assigned to a slide; or (ii) the wrong reagent is applied to a slide. Prior art devices, such as the staining device in Stark reference, relied on manually determining whether a particular slide would undergo a particular staining sequence. Specifically, prior to execution of a staining run, staining sequences were programmed for certain slide positions (e.g., position 1 was

programmed with sequence 1, position 2 was programmed with sequence 2, etc.). The operator was thus required to manually place the slide in the predetermined slide position in order to ensure the proper staining protocol (e.g., for staining sequence 2, the operator was required to insert the slide in position 2). Likewise, the prior art, including the Stark reference, relied on manually identifying the reagent container. Specifically, prior to beginning a staining run, the necessary reagents for the staining run were assigned to particular reagent container positions (e.g., position 1 was programmed with reagent 1, position 2 was programmed with reagent 2, etc.). The operator was thus required to insert the proper reagent container in the proper position. By contrast, the automatic identification of the staining protocols and the automatic identification of the reagent containers work in combination to eliminate the need for operator input at the beginning of a staining run. In turn, the present invention significantly reduces the possibility of applying the wrong staining sequence or applying the wrong reagent.

Complexity of the operation of the system

Another important consideration in a staining system is its complexity. Typically, laboratory technicians operate the staining devices and therefore do not wish to operate very complicated machinery. The automatic identification of the staining protocols and the automatic identification of the reagent containers work to significantly reduce the oversight necessary for operation of the system. The laboratory technicians need only insert the slides and the reagent containers into the staining system, and the system is able to process the slides. In contrast, the prior art required careful oversight, requiring the careful placement of the slides and the reagent containers in predefined places in turn requiring more thought in preparation for a staining run.

Ability to process more tissue samples without reprogramming

In a laboratory context, processing of large numbers of samples is important. However, prior art systems, such as Stark, assigned a particular staining protocol to a slide position. (e.g., position 1 was

programmed with sequence 1, position 2 was programmed with sequence 2, etc.). However, this type of arrangement is very inflexible, potentially resulting in failing to use all of the available positions in the slide support for staining. For example, if the technician wishes to stain all of the slides with sequence 1, yet slide position 2 is programmed with sequence 2, either the technician does not use slide position 2 or the programming must be changed. More than likely, the programming will not be changed, resulting in a failure to maximize the use of the staining module. With automated processing, the system is flexible. All of the positions in a staining system may be used during every run, thereby maximizing the ability to process tissue samples.

In summary, Stark et al., Sakurada and Saralegui et al. taken alone or in any combination, do not teach or suggest an apparatus having identification of the reagent container or determining whether reagent in the reagent container should be dispensed onto the slide in the particular configuration that Applicants particularly disclose and claim. Therefore, the Examiner has not established prima facie obviousness over Stark et al. in combination with Sakurada or Stark et al. in combination with Saralegui et al. and the rejections under 35 U.S.C. §103(a) should be withdrawn. Applicants respectfully request reconsideration of the application on that basis.

Rejection based on Stark, Sakurada, Saralegui and Rokugawa references

In Paragraph 10 of the Office Action, claims 84, 94-98 and 105-106 were rejected under 35 U.S.C. §103 as being unpatentable over Stark et al. (J. Immunol. Methods 107:89-92 (1988)) in view of Sakurada (U.S. Patent No. 4,346,056) or Saralegui et al. (U.S. Patent No. 5,439,645), and further in view of Rokugawa (U.S. Patent No. 4,844,868).

The Examiner asserted that the Stark, Sakurada and Saralegui references failed to recite the step of moving the reagent container and slide support relative to one another to position the reagent over the

slide. The Examiner further asserted that the Rokugawa reference teaches a method and apparatus for delivering reagents to reaction containers wherein a plurality of reagents 68 are supported on a reagent carousel 64 over a reaction carousel.

As discussed previously, the Stark, Sakurada and Saralegui references alone or in combination do not render the claims obvious. Likewise, the Rokugawa reference does not teach, or even suggest, the invention as claimed. The Rokugawa reference does not teach, or even suggest the use of bar coding, or any other form of automatic identification of the reagent containers or automatic determination of whether reagent should be dispensed onto the slide. In contrast, Claim 72 (upon which claim 84 ultimately depends) includes the limitations of “automatically identifying the reagent container using a computer” and “automatically determining whether reagent in the reagent container should be dispensed onto the slide”. Likewise, claim 94 includes the limitations of “automatically identifying the reagent container using a computer” and “automatically determining whether the reagent in the reagent container should be dispensed onto the slide”. Finally, claim 99 (upon which claims 105 and 106 ultimately depend) includes the limitation of “the bar code reader reads reagent bar codes on the reagent containers and wherein the reagent bar codes are sent to the computer for automatically identifying the reagent containers”. Therefore, applicants believe that the Stark, Sakurada, Saralegui and Rokugawa references, taken alone or in combination, do not render the claims obvious.



CONCLUSION

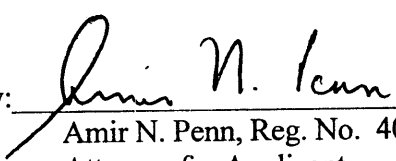
In view of the foregoing amendments and remarks, it is respectfully submitted that the presently pending claims in the application are believed to be in condition for allowance and patentably distinguish over the art of record. An early notice thereof is earnestly solicited.

Respectfully submitted,

McDonnell Boehnen Hulbert & Berghoff

Dated: June 26, 2001

By:


Amir N. Penn, Reg. No. 40,767
Attorney for Applicant

APPENDIX UNDER 37.CFR 1.121(c)

81. (Amended) The method of claim 80 wherein the slide bar code identifies a slide sample placed on the slide and identifies a [histochemical process] sequence of reagents for the slide sample.

88. (Amended) A method of dispensing reagents onto a slide, the method comprising the steps of:

providing a plurality of reagent containers in a reagent support, each of the reagent containers having a reagent barcode;

providing slides on a slide support, the slides having at least one slide bar code;

providing a bar code reader;

reading the bar codes on the reagent containers;

determining reagents in the reagent containers based upon the reading of the bar codes on the reagent containers;

reading the slide bar [code] codes on the slides;

determining a sequence of reagents to be applied on the slides based upon the reading of the slide bar [code] codes on the slides; and

dispensing the reagents in the reagent containers based upon the sequence of reagents to be applied.

94. (Amended) A method of dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container;

providing at least one slide on a slide support;

automatically identifying the reagent container using a computer;

automatically determining whether the reagent in the reagent container should be dispensed onto the slide;

moving the reagent container and the slide support relative to one another to position the reagent container over the slide; and

dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide.

99. (Amended) An automated biological staining apparatus comprising:

a slide support for holding slides;

slide support drive means for moving the slide support;

a reagent tray for supporting reagent containers;

reagent drive means for moving the reagent tray;

bar code reader;

reagent dispensing device for applying reagent onto a particular slide; and

computer in communication with the slide support drive means, the reagent drive means, [and] bar code reader and means for dispensing reagent,

wherein the bar code reader reads reagent bar codes on the reagent containers and slide bar codes on the slides, and

wherein [the reagent bar codes are sent to] the computer [for] automatically [identifying] determines whether reagent in the reagent containers should be dispensed onto the particular slide.

103. (Amended) The automated biological staining apparatus of claim 99 wherein the bar code reader reads slide bar codes on the slides and wherein the slide bar codes are sent to the computer for automatically determining whether reagent in the reagent containers should be dispensed onto the [slides] particular slide.

104. (Amended) The automated biological staining apparatus of claim 99 further comprising:

homing device connected to the slide support and in communication with the computer wherein the homing device determines position information for the [slides] particular slide.

105. (Amended) The automated biological staining apparatus of claim 99 wherein the computer controls the movement of [moves] the reagent tray and the slide support to move relative to one another to position the reagent containers over the [slides] particular slide.

107. (Amended) An automated biological staining apparatus comprising:

a slide support for holding slides;

slide support drive means for moving the slide support;

a reagent tray for supporting reagent containers;

reagent drive means for moving the reagent tray;

means for automatically identifying the reagent containers;

means for automatically determining whether reagent in the reagent containers should be dispensed onto [the] a particular slide; and

reagent dispensing device for applying reagent onto a particular slide

[computer in communication with the slide support drive means, the reagent drive means, the means for automatically identifying the reagent containers, and the means for automatically determining whether reagent in the reagent containers should be dispensed onto the slide].

109. (Amended) The automated biological staining apparatus of claim 107 wherein means for automatically determining whether reagent in the reagent containers should be dispensed onto the slide includes a bar code reader, wherein the bar code reader reads slide bar codes on the slides and wherein the slide bar codes are sent to the computer for automatically determining whether reagent in the reagent containers should be dispensed onto the [slides] particular slide.

EXHIBIT

6



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
 Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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09/452,309 12/01/99 COPELAND

K 97.008-U

EXAMINER

020306 IM52/0813
 McDONNELL BOEHNNEN HULBERT & BERGHOFF
 300 SOUTH WACKER DRIVE
 SUITE 3200
 CHICAGO IL 60606

BEY, P
 ART UNIT PAPER NUMBER

1743
 DATE MAILED:

08/13/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No.		Applicant(s)	
	09/452,309		COPELAND ET AL.	
	Examiner		Art Unit	
	P. K. Bex		1743	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on 27 June 2001.

2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) 72-113 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5) ☒ Claim(s) 88-93 and 99-113 is/are allowed.

6) ☒ Claim(s) 72-79, 84-87 and 94-98 is/are rejected.

7) ☒ Claim(s) 80-83 is/are objected to.

8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some * c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. _____.

3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15) ☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)	4) <input type="checkbox"/> Interview Summary (PTO-413) Paper No(s). _____
2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)	5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152)
3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO-1449) Paper No(s) <u>10</u> .	6) <input type="checkbox"/> Other:

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DETAILED ACTION

Claim Rejections - 35 USC § 103

1. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

2. The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

3. Claims 72-79 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stark *et al* (*J. Immunol. Methods* 107:89-92 (1988)) in view of Sakurada (USP 4,346,056).

Stark *et al* disclose an automatic method and apparatus for immunocytochemistry. The apparatus comprises a rotatable slide support carousel for mounting a plurality of slide supports 7, drive means 4 for rotating the slide support carousel, and reagent containers 3 for applying a predetermined quantity of reagent into a slide (Fig. 1). Stark *et al* fail to specifically recite sensors for detecting positions of slide samples or reagent containers, or a bar code reader for identifying types of reagents.

Sakurada disclose the rotation position of the reagents through the use of a detector 58 which is composed of photocouplers. The lowest photocoupler is used to deliver an original

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point for indexing the reagent vessel holder. This allows the desired reagent vessel to be moved to the a desired position along the circumference of the holder. Moreover, Sakurada teach the use of reagent vessels each comprising a label 33 (Fig. 3) corresponding to the different types of reagents used, determined automatically by a detector 31. The detectors 31 and 58 are both in communication with a control device 24 thereby providing "automatic" control (column 3, lines 20-31, column 4 line 3- column 6, line 7, Figs. 3, 5). Note: the term "automatic" is well known in the art to mean, the ability to act or operate in a manner essentially independent of external influence or control.

Such a delivery control mechanism is considered well known for its reliable functions, speed of operations in the autoanalyzer art. Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have provided the modified apparatus of Stark *et al* with sensors for detection and identification of containers and its movements, as taught by Sakurada, in order to carry out the sample analysis automatically, reliably and simply. The use of bar codes to automatically convey information to a control allows all the information relating to a new experiment to be incorporated into the bar code without the need to reprogram the chemical analyzer itself.

4. Claims 84-87 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stark *et al* (*J. Immunol. Methods* 107:89-92 (1988)) in view of Sakurada (USP 4,346,056) as applied to claim 72 above, and further in view of Rokugawa (USP 4,844,868).

Stark *et al*, and Sakurada as previously discussed, fail to specifically recite the step of moving the reagent container and slide support relative to one another to position the reagent container over the slide. Rokugawa teaches a method and apparatus for

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delivering reagents to reaction containers wherein a plurality of reagents 68 are supported on a reagent carousel 64 which is positioned over a reaction carousel (Fig. 1). Such an arrangement of reagent delivery system would provide an improved automatic chemical analysis with which it is possible to eliminate dead space in the reagent passage, reduce size, reduce distribution time and eliminate cross contamination (column 1, lines 38-43). Note: the reagent carousels of Rokugawa would inherently include homing and indexing means in order to identify each position of reagent containers and rotate an appropriate reagent container to the reagent dispensing zone which is positioned under the plunger mechanism 100, since different reagents are used in the Rokugawa system.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have provided the apparatus of Stark *et al* and Sakurada with a reagent distributor as taught by Rokugawa, in order to provide an improved automatic chemical analysis with which it is possible to eliminate dead space in the reagent passage, reduce size, reduce distribution time and eliminate cross contamination.

5. Claims 94-98 are rejected under 35 U.S.C. 103(a) as being unpatentable over Heidt *et al* (USP 5,089,229) in view of Sakurada (USP 4,346,056).

Heidt *et al* teach a chemical analyzer a method of dispensing a fluid onto a slide providing one metering means 16 positioned above a slide carousel 50. The metering device includes a pipette assembly which holds a certain amount of the fluid in the tip. The method of dispensing the fluid onto the slide 71. Each slide on the carousel includes a bar code 86 on the upper surface. The bar code includes information concerning the analyte contained thereon and when interfaced with an optical code reader 158 provides this information to the computer and

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determines what test are to be performed (column 15, lines 9-24) Additionally, Heidt *et al* teach whether the fluid from pipette should be dispensed based on detectors 218 which based on disturbance in the light beam automatically determines whether a test slide has been properly positioned below the tip of the pipette. Heidt *et al* teach a computer into which the fluid to be dispensed is entered. However, Heidt *et al* do not disclose a reagent identifying means to automatically identify the reagent via a computer. However, such a method of automatically identification is considered conventional in the art, see Sakurada. Sakurada teach the use of reagent vessels each comprising a label 33 (Fig. 3) corresponding to the different types of reagents used, determined automatically by a detector 31.

Accordingly, it would have been obvious to one of ordinary skill in the art to have included in the chemical analyzer of Heidt *et al* the method of automatic detection as taught by Sakurada. Such an method of automatic detection would eliminate any possibility of data entry errors which are common when information is manually entered into a computer.

Response to Arguments

6. Applicant's arguments filed June 27, 2001 have been fully considered but they are not persuasive. Applicant argues that Stark *et al* do not teach "automatically" identifying the reagent container or automatically determining whether a reagent in the reagent container should be dispensed onto a slide. However, Examiner points out that the device of Stark *et al* is under the control of software which was written before operation or stored procedure, therefore it provides "automatic" control since it is the able to act or operate in a manner essentially independent of external influence or control of an operator at the time of use. Additionally, whether or not the

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proper positions of the reagents or slides are based on preprogramming the computer is not relevant since the apparatus does not require manual operation *during* the procedure.

Allowable Subject Matter

7. Claim 88-93 and 99-113 are allowable.
8. Claims 80-83 objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

The following is a statement of reasons for the indication of allowable subject matter: the instant claims are drawn to an automated slide staining apparatus and methods. While numerous slide staining apparatus and methods exist, none of the prior art teaches or suggest providing a plurality of reagent containers in reagent support wherein each of the reagent container have a reagent barcode. The apparatus also providing slides on a slide support, each of the slides having at least one slide bar code. Wherein the apparatus provides a barcode reader for reading the bar codes on the reagent containers, determining reagents in the reagent containers based on the reading of the bar codes. Thereafter, the barcode reader reading the slide bar codes and determining the sequence of reagent to be applied on the slides base upon the reading of the slide bar codes. Lastly, dispensing reagents in the reagent containers based upon the sequence of reagents to be applied.

Conclusion

9. 72-79, 84-87 and 94-98 are rejected. Claim 80-83 are objected. Claims 88-93 and 99-113 are allowable.

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10. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure are Shah *et al*, Kanamori *et al*, and Ushikubo. They are cited of interest in that they show various methods and apparatus for dispensing reagents into a slide or container.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

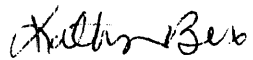
11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to P. Kathryn Bex whose telephone number is (703) 306-5697.

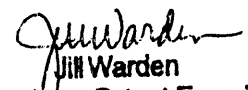
The fax number for the organization where this application or proceeding is assigned is (703) 305-7718 for official papers prior to mailing of a Final Office Action. For official papers after mailing of a Final Office Action, use fax number (703) 305-3599. For unofficial or draft papers use fax number (703) 305-7719. Please label all faxes as official or unofficial. The above fax numbers will allow the paper to be forwarded to the examiner in a timely manner.

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Art Unit: 1743

Page 8

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0661.


P. Kathryn Bex
Patent Examiner
AU 1743
8/7/01


Jill Warden
Supervisory Patent Examiner
Technology Center 1700

Notice of References Cited	Application/Control No. 09/452,309	Applicant(s)/Patent Under Reexamination COPELAND ET AL.	
	Examiner P. K. Bex	Art Unit 1743	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification	
	A	US-4935875	06-1990	Shah et al.	364	497
	B	US-5356595	10-1994	Kanamori et al.	422	65
	C	US-5424036	06-1995	Ushikubo	422	64
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification	
	N						
	O						
	P						
	Q						
	R						
	S						
	T						

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

EXHIBIT

7

D. Lawrence
#1613
8.29.01

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
 (Case No. 97,008-U)

In re Application of:)	
)	
COPELAND, et. al)	
)	Group Art Unit: 1743
Serial No.: 09/452,309)	
)	Examiner: Bex, P.
Filed: December 1, 1999)	
)	
For: Automated Biological)	
Reaction Apparatus)	

Commissioner for Patents

Washington, DC 20231

DO NOT ENTER

OK TO ENTER

OK AS ENTERED

Dear Sir:

AMENDMENT IN RESPONSE TO FINAL OFFICE ACTIONDATED AUGUST 13, 2001

TECHNOLOGY CENTER 1700

AUG 28 2001

RECEIVED

Applicants hereby respond to the final Office Action dated August 13, 2001.

IN THE CLAIMS:

Please cancel claims 72-79, 84-87 and 94-98 without prejudice. Please amend claim 80 as follows. A marked up version of the amended claim, to show all the changes, is attached hereto on pages separate from the amendment in accordance with 37 CFR 1.121(c)(1)(ii).

~~80~~. (Amended) A method of dispensing reagents onto a slide, the method comprising the steps of:

- providing at least one reagent container;
- providing at least one slide on a slide support;
- automatically identifying the reagent container using a computer;
- automatically determining whether reagent in the reagent container should be dispensed onto the slide; and

dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide,

wherein the step of automatically determining whether reagent in the reagent container should be dispensed onto the slide includes the steps of:

providing a bar code reader;

reading a slide bar code placed on the slide using the bar code reader thereby acquiring slide information, the slide information indicating reagents to be applied to the slide; and

sending the slide information to the computer.

REMARKS

Claims 72-79, 84-87 and 94-98 are cancelled herewith without prejudice. Claim 80 is written in independent form. Claims 80-83, 88-93 and 99-113 are in the application for consideration.

Claim Rejections – 35 U.S.C. §103

In Paragraphs 3, 4, and 5 of the Office Action, claims 72-79, 84-87 and 94-98 were rejected under 35 U.S.C. §103(a) as being obvious. In order to advance prosecution, Applicants hereby cancel claims 72-79, 84-87 and 94-98 without prejudice in order to prosecute these claims in a continuation application.

Allowable Subject Matter

In paragraph 7, the Office Action stated that claims 88-93 and 99-113 are allowable. In Paragraph 8 of the Office Action, claims 80-83 were objected to as being dependent on a rejected base claim, but would be considered allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. Applicants hereby rewrite claim 80 in

independent form, including all of the limitations of independent claim 72. Claims 81-83 each ultimately depend on claim 80.

CONCLUSION

In view of the foregoing amendments and remarks, it is respectfully submitted that the presently pending claims in the application are believed to be in condition for allowance and patentably distinguish over the art of record. An early notice thereof is earnestly solicited.

Respectfully submitted,

McDonnell Boehnen Hulbert & Berghoff

Dated: 8/24/01

By: Amir N. Penn
Amir N. Penn, Reg. No. 40,767
Attorney for Applicant

APPENDIX UNDER 37 CFR 1.121(c)

80. (Amended) A method of dispensing reagents onto a slide, the method comprising the steps of: [The method of claim 72]

providing at least one reagent container;

providing at least one slide on a slide support;

automatically identifying the reagent container using a computer;

automatically determining whether reagent in the reagent container should be dispensed onto the slide; and

dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide,

wherein the step of automatically determining whether reagent in the reagent container should be dispensed onto the slide includes the steps of:

providing a bar code reader;

reading a slide bar code placed on the slide using the bar code reader thereby acquiring slide information, the slide information indicating reagents to be applied to the slide; and

sending the slide information to the computer.

EXHIBIT

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JIM 04645

An automated device for immunocytochemistry

Erwin Stark¹, Ditmar Faltinat² and Ruediger Von der Fecht²¹ *Division of Neurology and Clinical Neurophysiology, Hannover Medical School, Hannover, F.R.G.,*
and ² *Central Division for Research Instrumentation, Hannover Medical School, Hannover, F.R.G.*

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A microprocessor controlled device for automation of immunoenzyme histological and cytological staining procedures is described. As the sequence of processing is controlled by a computer program, the apparatus is flexible and the possibility of technical errors is reduced.

Key words: Automation; Immunoenzyme technique; Immunocytochemistry

Introduction

Immunocytochemical methods have made great progress during the last decade and a great number of interstitial, surface membrane and intracellular antigens now can be visualised. The availability of monoclonal antibodies on the one hand and of enzyme-anti-enzyme complexes on the other hand have made this possible. However, when monoclonal antibodies to antigens of low density are used, it is difficult to enhance the strength of the immunocytochemical staining without increasing the background staining. One solution is to use an enzyme-anti-enzyme procedure and to incubate two or more times with the second and third antibody. The disadvantages of these techniques are: (i) the long time periods involved; (ii) with the number of incubation steps increasing, the probability of incorrect processing rises. By shortening the time of incubation the total processing time may be reduced, but the number of slides one laboratory assistant can treat per day

is not increased. The more frequent the steps, the fewer the number of slides that can be processed in a batch. In order to facilitate automated processing in immunocytochemistry a machine has been developed.

Materials and methods

The device

In order to perform automated immunocytochemical processing a microprocessor controlled device has been designed. The principle is shown in Fig. 1. On the microscope slides, the area containing the cells or tissue is bordered by a circle consisting of water-resistant varnish. The slides are clamped on a revolving table, which is rotating in a plastic tub. In order to retain moisture and prevent drying, the tub and revolving table are enclosed in a plastic case. The table is driven directly by an electronically controlled stepper motor (0.9° increments). The zero position of the motor and table is encoded by a coded disk with opto-electronic scanning. The antibody solutions are pipetted onto the slides by standard plastic syringes with rubber pistons. Each syringe is moved by a bar, which is driven by a DC motor

Correspondence to: E. Stark, Neurol. Klinik mit klin. Neuropathologie – OE 7210, Med. Hochschule Hannover, Konstanty-Gutschow-Str. 8, 3000 Hannover 61, F.R.G.

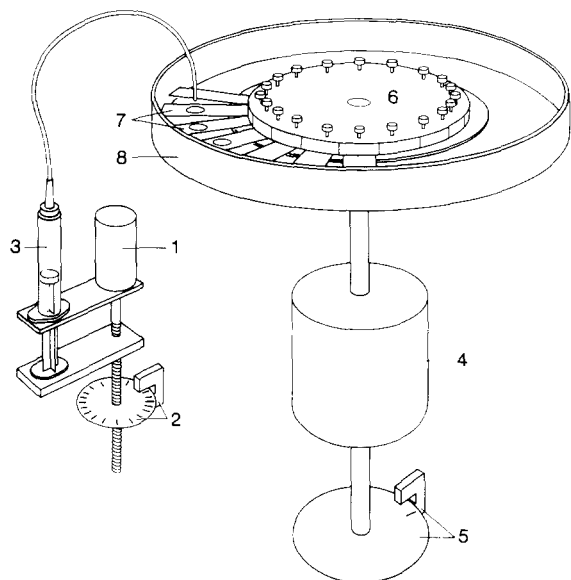


Fig. 1. Schematic drawing of the device. (1) DC motor with spindle for driving a bar. (2) Coded disk for volume scanning. (3) Disposable syringe. (4) 0.9° stepper motor. (5) Opto-electronic scanner. (6) Revolving table as slide carrier. (7) Maximal 20 standard slides (26×76 mm). (8) Rinsing tub. To simplify matters only one of five pipetting units (parts 1–3) is shown.

via a spindle and volume control is achieved by opto-electronic scanning. In order to take off the antibodies, the rotation table is accelerated to high

speed by driving the stepper motor at maximum rate. Any antibody solution remaining on the slides and in the tub is washed away by pumping buffer into the tub. A volume of about 500 ml is necessary to ensure adequate covering of all slides. The filling and emptying of the tub is controlled by thermoelectric level detectors. As the volume in the tub is higher than in containers used for manual processing the number of washing steps is reduced. The apparatus is controlled by a modular microprocessor system with standard boards (Siemens SMP system).

The device has been tested using cyto-spin preparations of CSF cells (Feller and Pawaresch, 1983) and of blood mononuclear cells obtained by density gradient centrifugation. In addition some paraffin section slides have been processed, using, firstly, a two-step indirect immunoperoxidase method (Stark and Wurster, 1987) and, secondly, a double alkaline phosphatase-anti-alkaline phosphatase (APAAP) procedure (Cordell et al., 1984). Three different, commercially available, APAAP complexes (Zymed, San Francisco; Dakopatts, Hamburg; Dianova, Hamburg), have been tested. In the APAAP procedure stored frozen substrate solution was tested in order to investigate further the potential of the machine. Step 1 comprised 2 mg naphthol AS-MX phosphate dissolved in 0.2 ml dimethylformamide and 8.8 ml Tris buffer (pH 8.2, 0.1 M) and step 2 used a solution of 10 mg

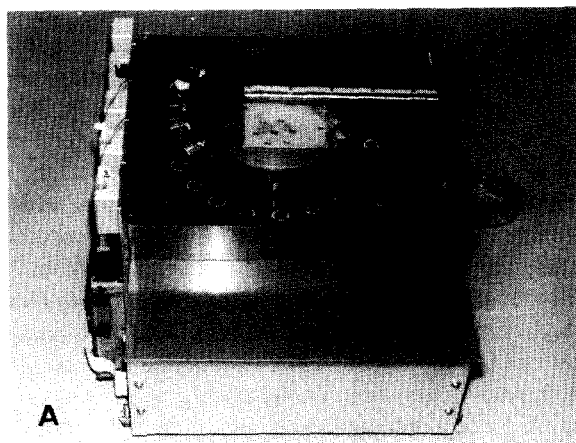


Fig. 2. Front (A) and left side (B) view of the device.

fast red TR salt in 1 ml distilled water. Both solutions had been stored frozen at -18°C . Immediately before use they were thawed in warm water, mixed and filtered.

Automatically processed immunostaining of slides was compared to manually processed slides.

The control program

The software to control the device was written in Assembler and the dialogue with the operator was via a standard terminal. At the start of the program the actual processing sequence could be programmed individually or, alternatively, this part of program could be deleted and a previously stored procedure requested. Up to six staining procedures could be held in an accumulator buffered memory. For each specimen the number of slides and the names of the primary antisera were entered. The slides were inserted one after the other and the table advanced one position by actuating a pedal switch. When all of the slides were inserted, the program requested that the pipetting units with the syringes be filled with the appropriate antibody solutions. Then the application of the primary antibody was requested. In order to avoid errors the name of the antibody was indicated on the screen and all slides designated to receive this antibody were successively moved to the window in the lid of the device. When all of the slides were covered by antisera, incubation was permitted to occur for a predetermined time. Then buffer was pumped into the tub and after a short revolution of the table at low speed the buffer was pumped out; drops remaining on the slides were removed by a further centrifugation step. The washing cycle was repeated as often as programmed. Then the first slide was moved to the outlet of the second syringe and the prescribed volume pipetted onto the slides. All slides were successively treated in this way and the processing completed by applying all further reagents in this manner. If substrate solutions of limited stability were used, they were inserted into the syringe immediately before use. When this mode was selected, the program requested insertion of this syringe after the last antibody had been applied. The software to control the device is programmable and may be easily adapted to other immunocytochemical procedures.

Results

No matter which procedure or antibodies were used with the automatic device, the quality and reliability of immunostaining of blood or CSF cells and paraffin sections were identical or superior to the results obtained by manual processing. To date approximately 1000 slides have been processed by the device and errors in processing resulting in slides which could not be evaluated have been very rare. If the device was programmed to deliver approximately $80\text{ }\mu\text{l}$ of the diluted antibody on each slide, the area containing the cells was sufficiently covered. Using a double-bridge APAAP technique, the antibody could be diluted by a factor of three compared to a single-bridge APAAP technique. In order to ensure complete covering of the specimen the volume of antibody solution per slide has to be higher than the volume needed in manual processing.

The expenditure of work was considerably reduced and manual work was only necessary at the start and before the end of the staining procedure. In contrast to manual processing where a laboratory technician requires approximately 5 h to prepare 40 slides, with automated processing, the preparation time is reduced to one hour at the most. After an interval of 3–4 h there is a further period of activity lasting another 20 min. Even if the process was stopped by power failure, the prolonged incubation time did not appear to alter the staining results. Substrate solution frozen in two portions was stable for at least 2 weeks, and preparing substrate solution was only necessary once a fortnight.

Discussion

In conventional histo- and cytological staining procedures automated devices have been quite common for several decades. Today, devices for automatic staining give such reliable and reproducible results that their use is a necessary prerequisite when staining blood smears destined for automatic differential cell counting (Green, 1979). In another antibody-based technique, the enzyme-linked immunosorbent assay (ELISA), semiautomatic processing has given results of high preci-

sion (Sever, 1983) and ready automation is an important reason for the fast spread of this technique.

In immunocytochemistry relatively few aids for processing have been introduced. A heated incubation chamber is commercially available, and another complicated incubation chamber designed to reduce the amount of antibody needed to stain one specimen was recently described (Abbuhl and Velasco, 1985). An incubation chamber to facilitate immunocytochemistry of vibratome sections has also been described (Paull and King, 1983), but none of these devices can reduce the expenditure of manual work necessary for processing. Even if the expense of antibodies still restrict some of the wider applications of immunocytochemistry, this should, in our opinion, no longer present a handicap in the future. If the present trend continues, polyclonal antibodies will be successively replaced by monoclonal antibodies, which will in turn become less and less expensive.

However, the factor restricting immunocytochemistry will be the expenditure of labour, if automatic processing is not used. Until less expensive antibodies are available, it is possible, with the device developed, to reduce the total amount of antibody necessary for one slide. This can be done by multi-bridge enzyme-anti-enzyme procedures or by overnight processing with prolonged incubation times. There are only two disadvantages of automated processing. First, the amount of buffer necessary for rinsing is approximately five times larger than for manual processing. Second, the specimen to be processed must be placed at the same, predetermined position on each slide. If cell specimens are prepared by a cytocentrifuge this condition is fulfilled and for microtome sections it is useful to mark the slide, where the tissue sections should be placed.

In conclusion, automation in immunocytochemistry improves the results and increases the

number of samples that can be processed. In conjunction with more sophisticated techniques and diagnostic antibodies, it may enhance the acceptance of immunological methods in cytology and histology.

Acknowledgements

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EXHIBIT

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United States Patent [19]

Sakurada

[11] 4,346,056
[45] Aug. 24, 1982

[54] AUTOMATIC ANALYZING APPARATUS

[56]

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[73] Assignee: Olympus Optical Company Limited,
Tokyo, Japan

[21] Appl. No.: 177,446

Primary Examiner—Ronald E. Serwin
Attorney, Agent, or Firm—Stevens, Davis, Miller &
Mosher

[22] Filed: Aug. 11, 1980

[57] ABSTRACT

[30] Foreign Application Priority Data

Aug. 15, 1979 [JP] Japan 54/111525[U]
Aug. 17, 1979 [JP] Japan 54/112413[U]

An automatic analyzing apparatus comprising a plurality of reagent vessels adapted to be inserted into a reagent vessel holder and a detector for photoelectrically discriminating the reagent vessel holder in response to an analysis item necessary for examining an organic ability and the reagent vessels corresponding to different analysis items.

[51] Int. Cl.³ G01N 35/04
[52] U.S. Cl. 422/64; 422/67
[58] Field of Search 422/63, 64, 65, 67;
364/497

9 Claims, 10 Drawing Figures

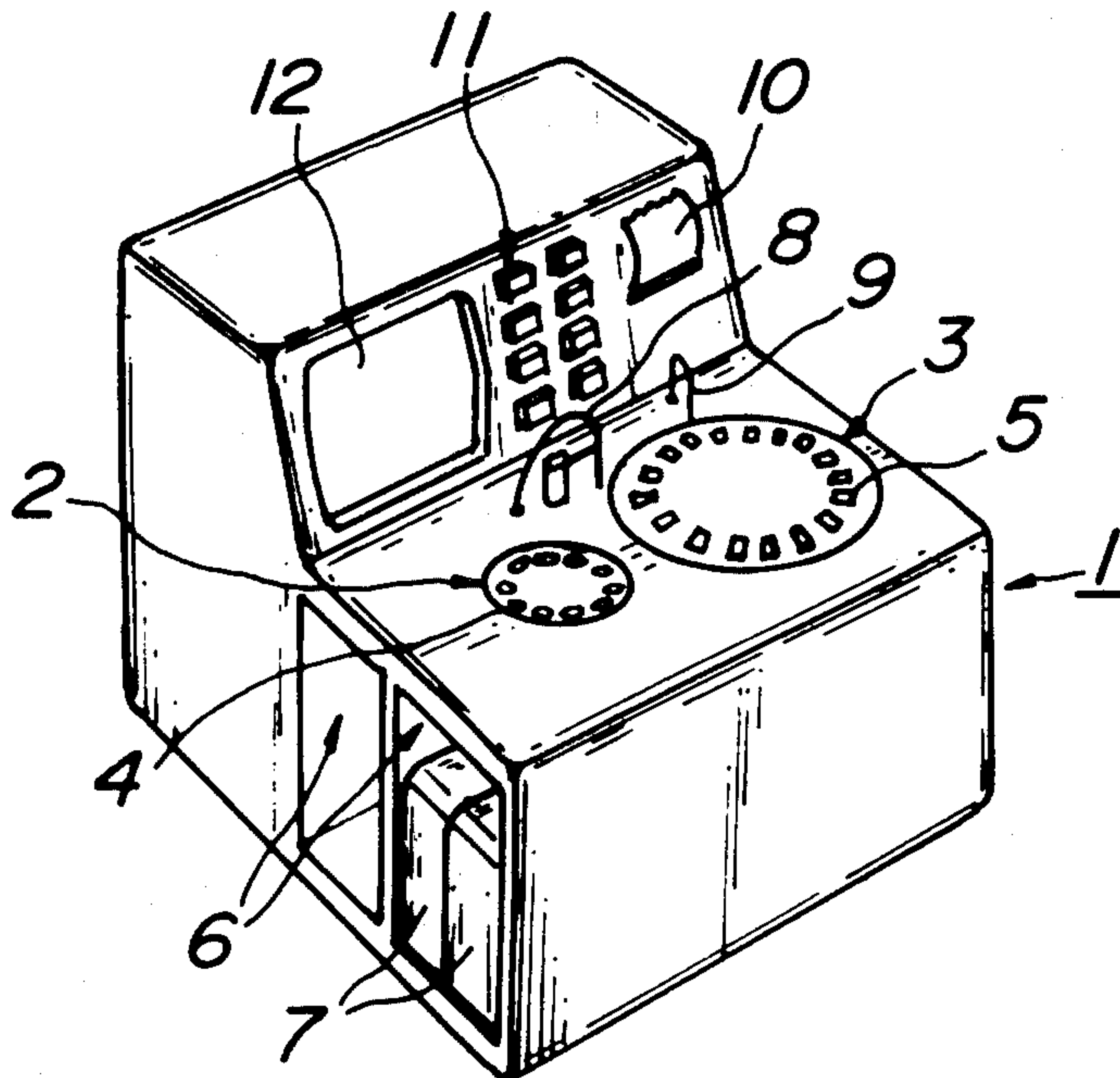


FIG. 1

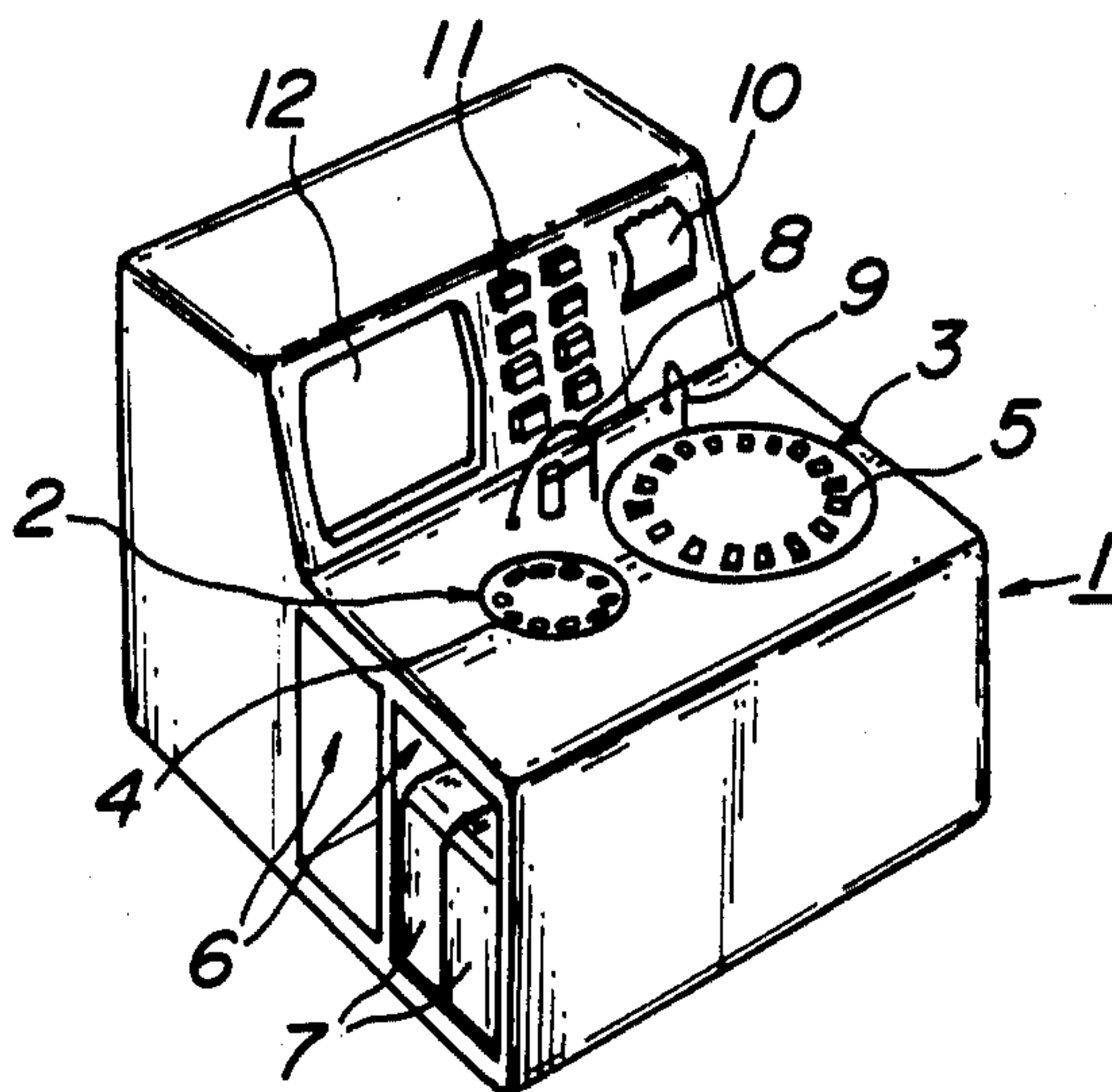


FIG. 3

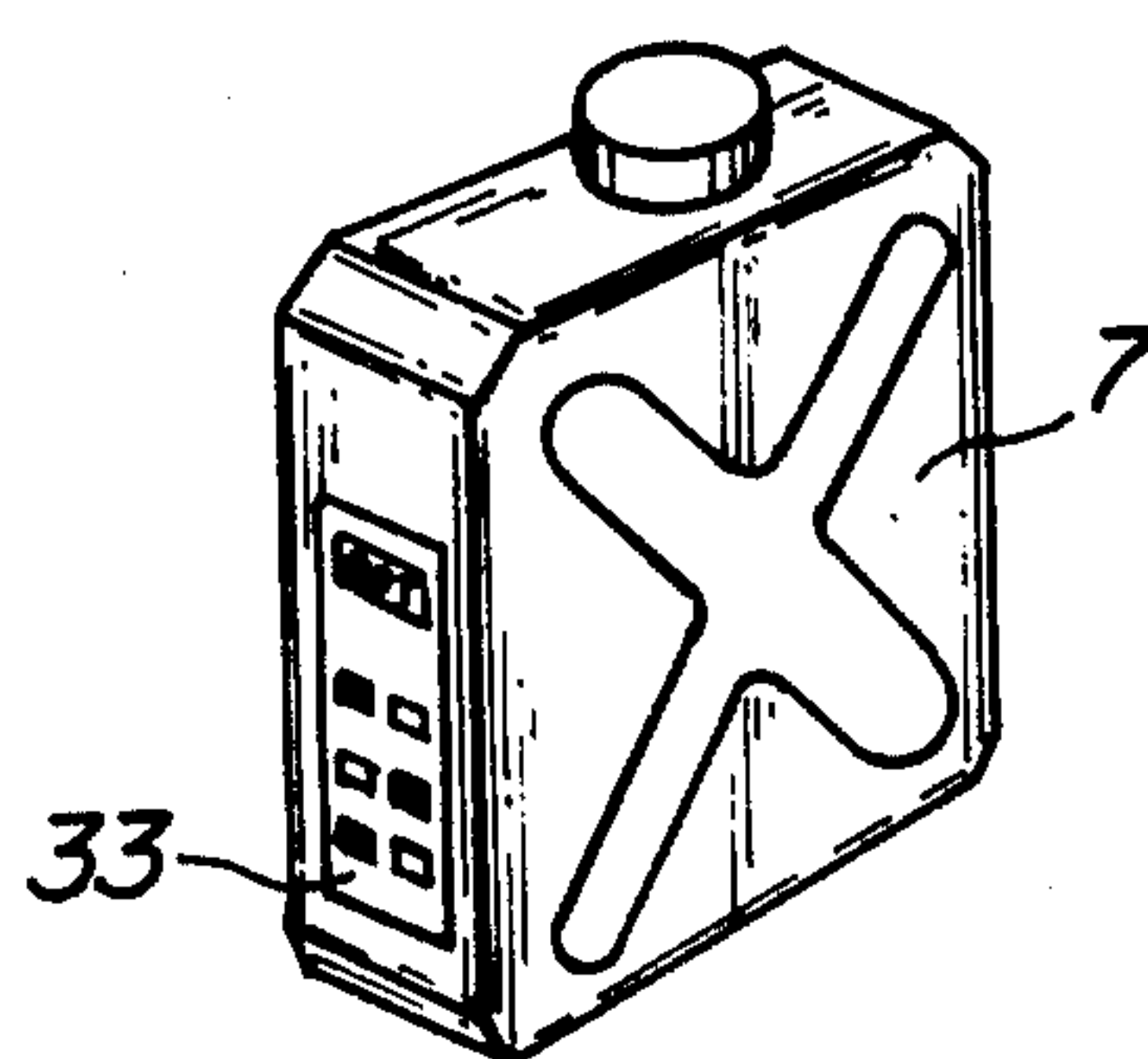


FIG. 2

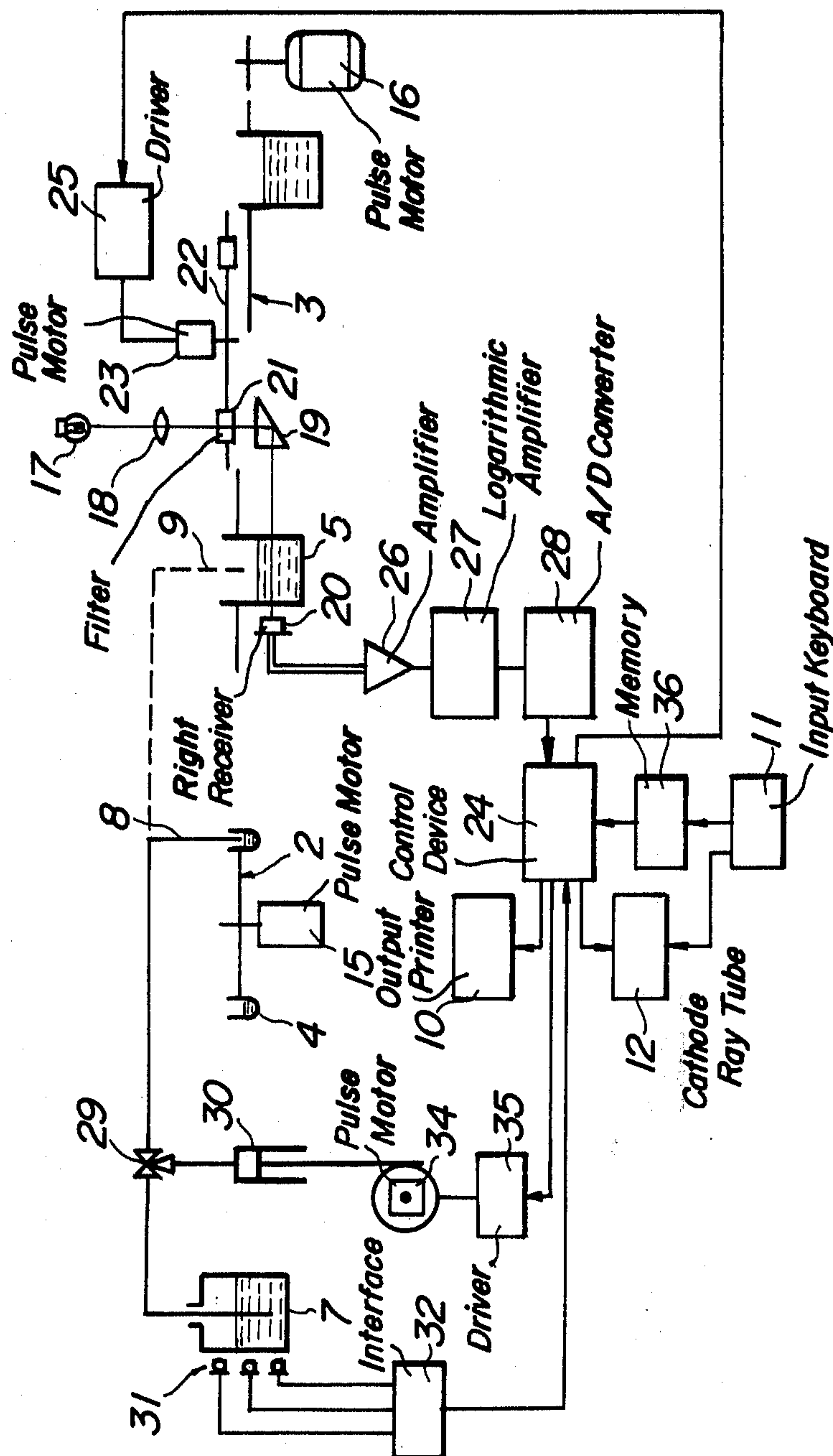


FIG. 4

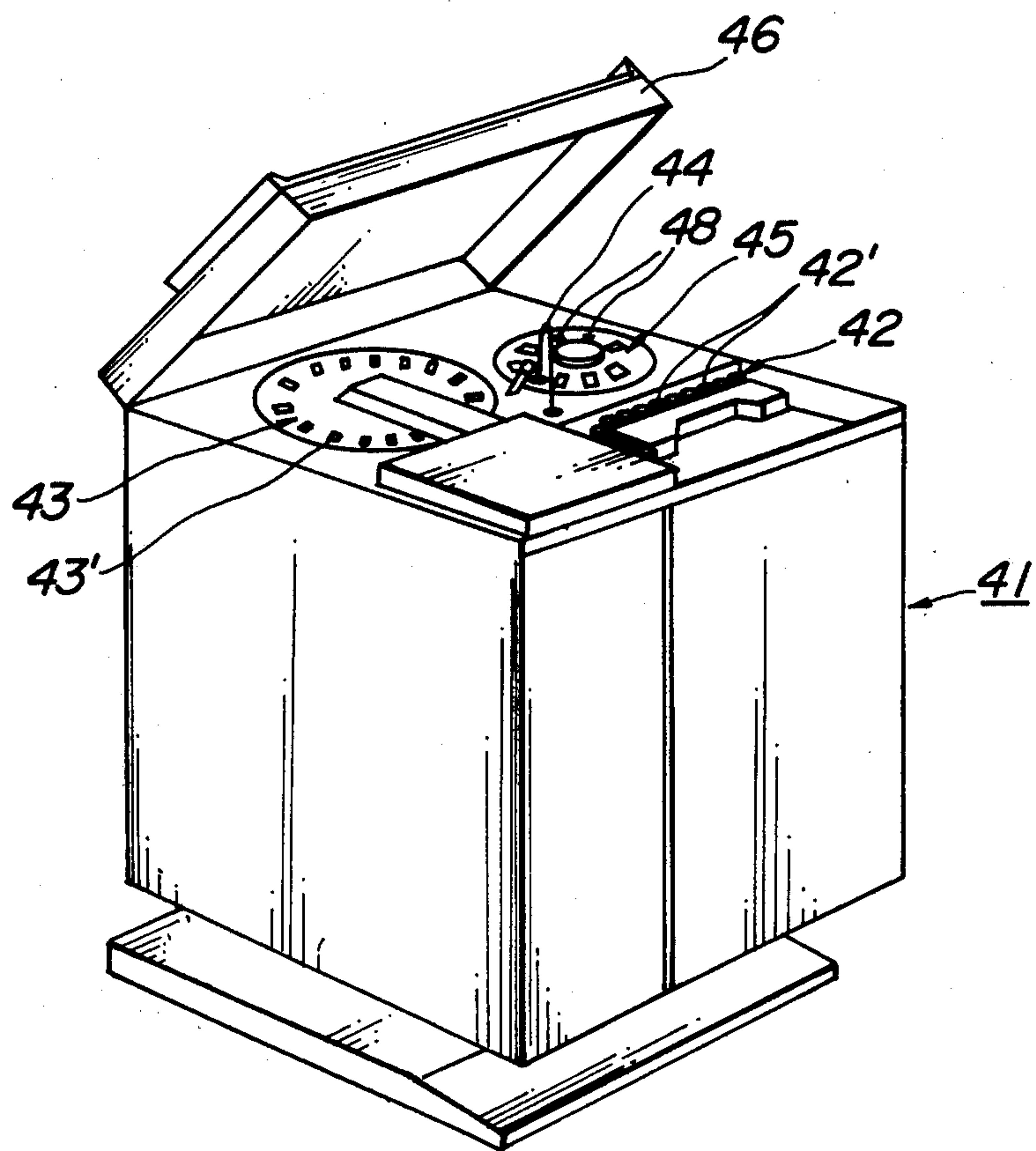


FIG. 5

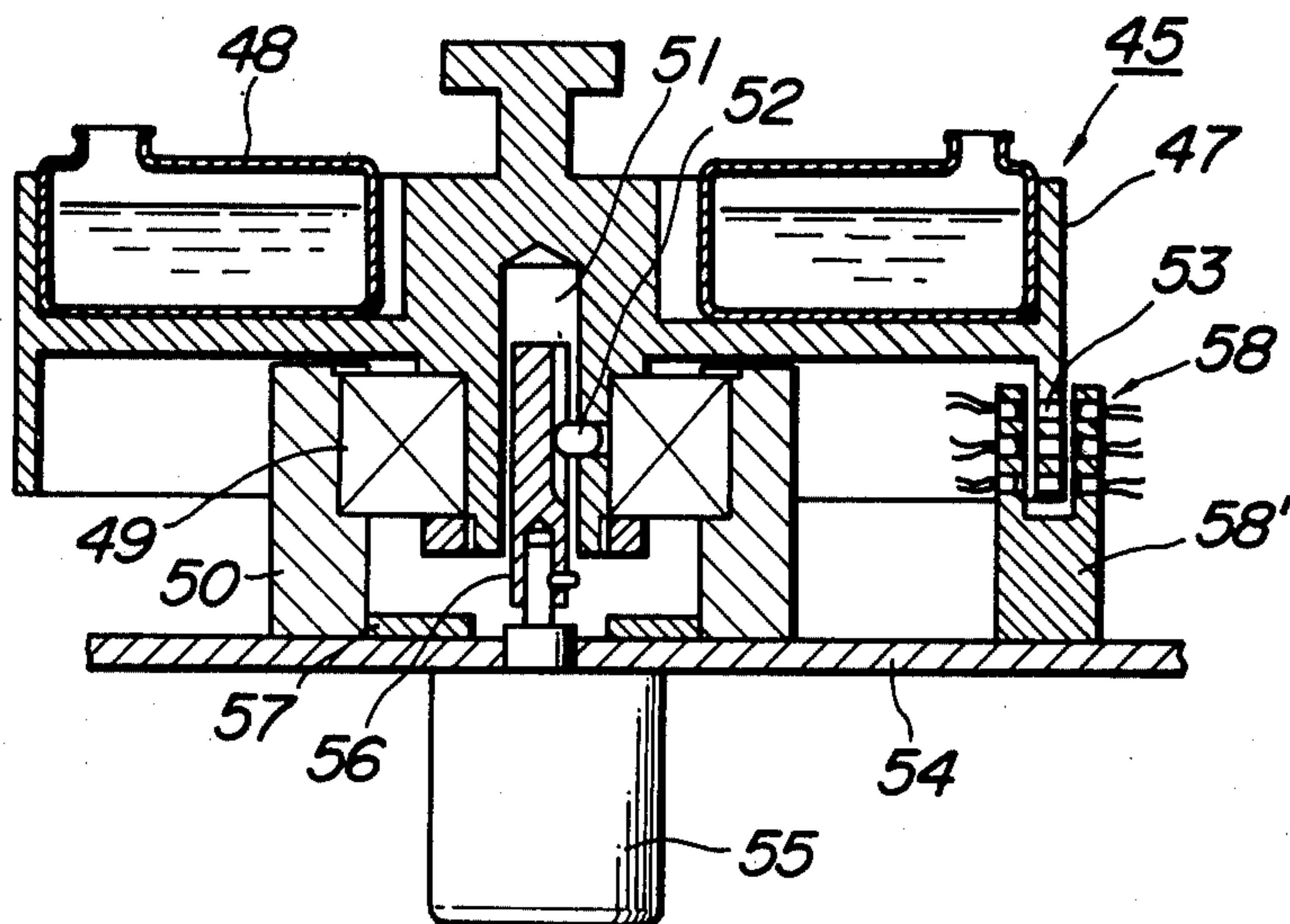


FIG. 6

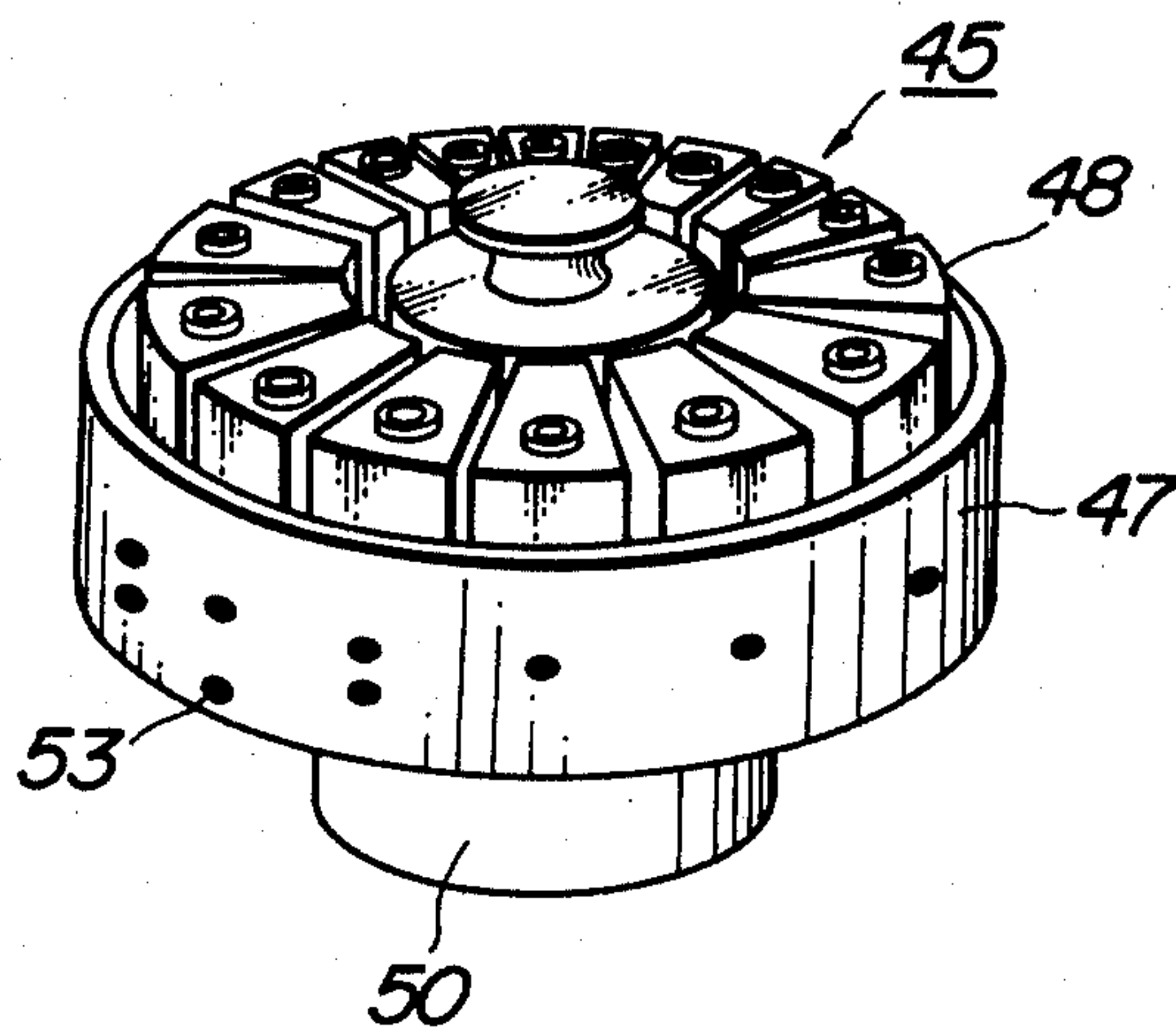


FIG. 7A

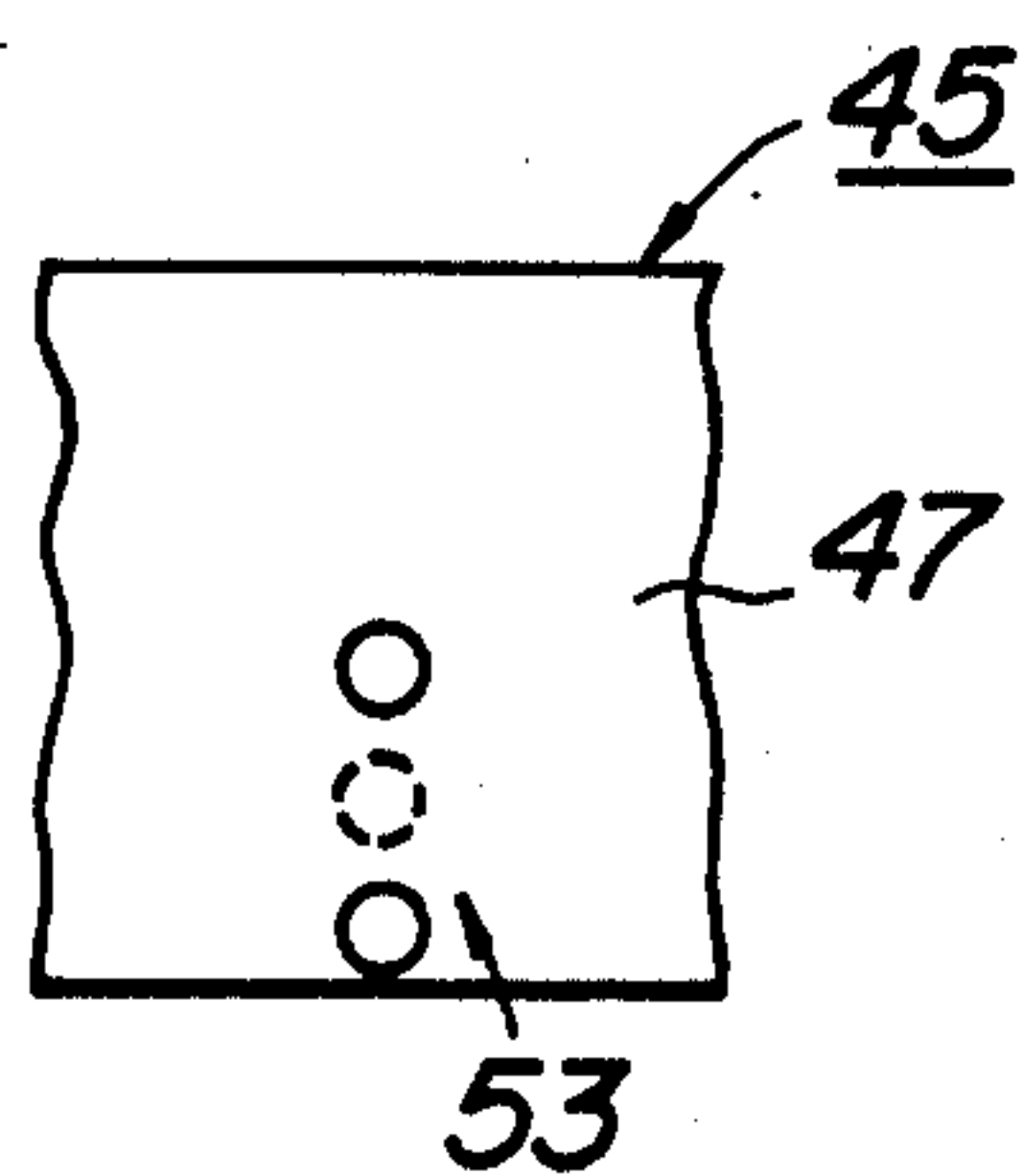


FIG. 7B

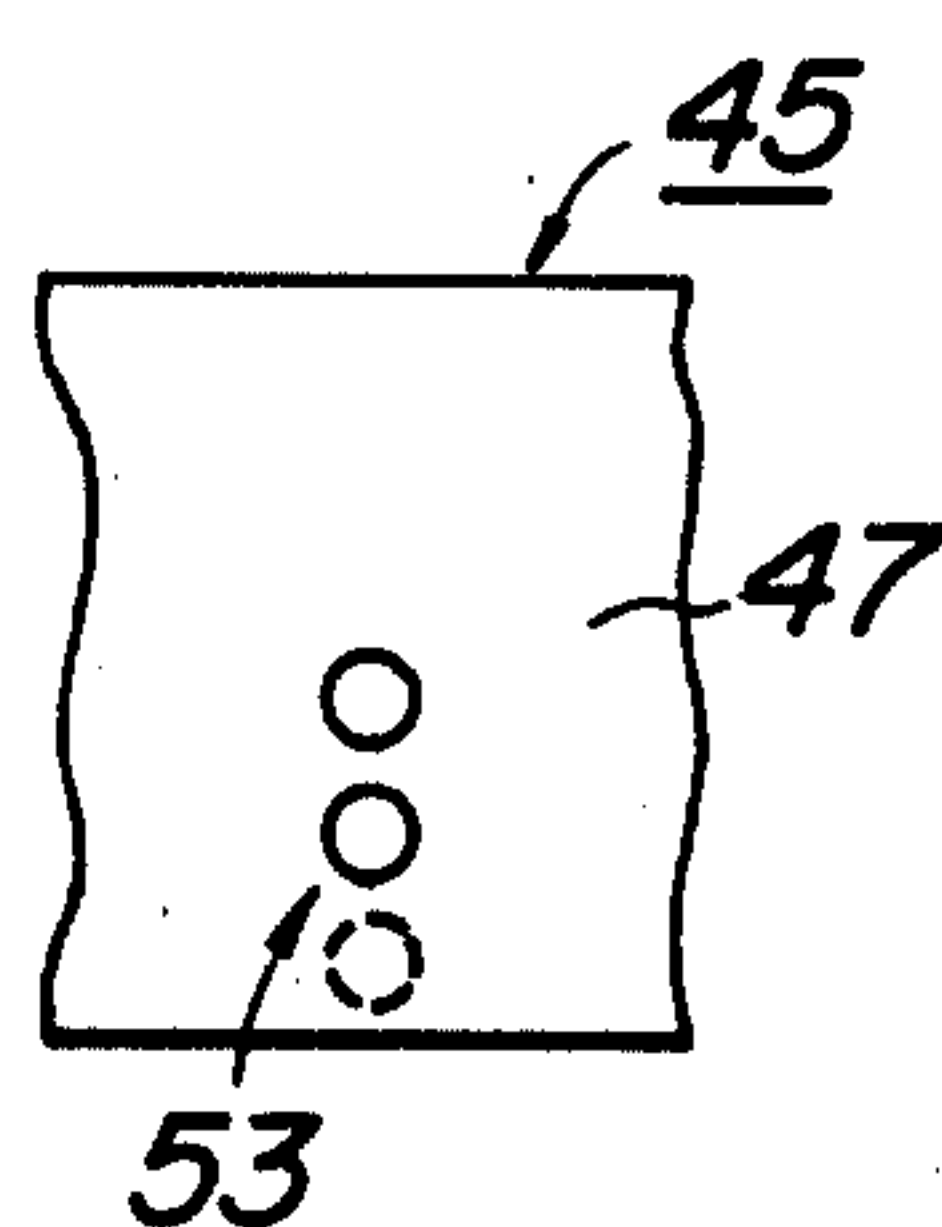


FIG. 7C

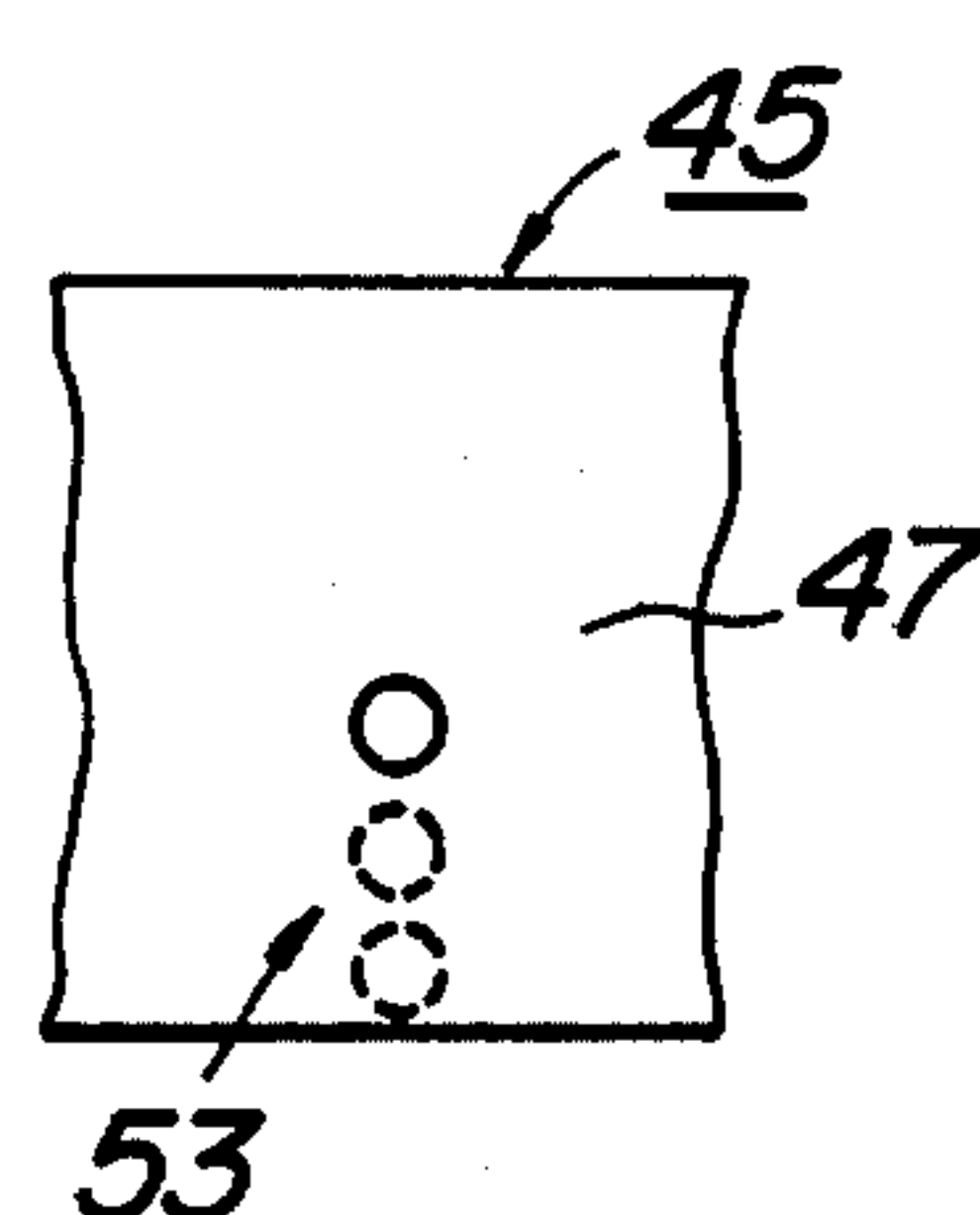
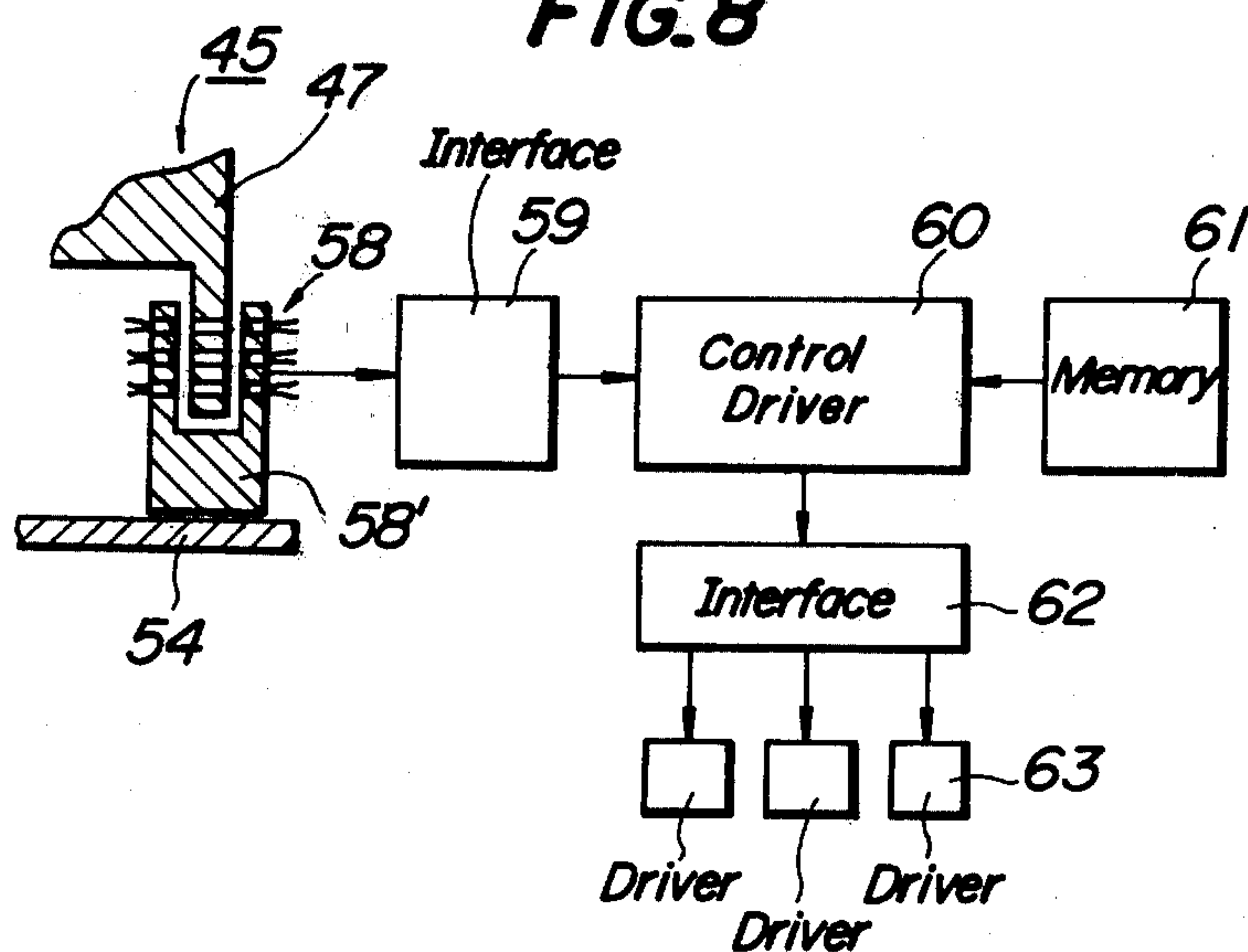


FIG. 8



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AUTOMATIC ANALYZING APPARATUS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to an automatic analyzing apparatus.

2. Description of the Prior Art

Various kinds of automatic analyzing apparatuses, in which a collected sample such as serum, urine or the like and a reagent necessary for analyzing a desired composition contained in the sample are injected into a reaction cuvette to prepare a test liquid and the test liquid is subjected to a colorimetric measurement so as to effect a quantitative analysis on the desired composition contained in the sample, have heretofore been proposed. But, in such conventional automatic analyzing apparatus, when an analysis item is set an operator is generally required not only to effect an operation of setting a reagent to be used, but also to effect an operation of setting various conditions in response to analysis items such as an amount of sample to be injected, amount of reagent to be injected, photometric wave length, or the like or effect an operation of calling out the above mentioned analysis conditions in response to the analysis items which have been supplied as inputs beforehand. As a result, the conventional automatic analyzing apparatus has the disadvantage that it is complex in operation and that there is a risk of the erroneous operation being induced.

Meanwhile, the automatic analyzing apparatus can generally analyze at least 30 items, whereas desired average number to be analyzed for one sample is on the order of 6 to 8. As a result, the conventional automatic analyzing apparatus is constructed such that it can simultaneously analyze 6 to 12 channels, that is, 6 to 12 analysis items. In such automatic analyzing apparatus, if it is desired to analyze more than 12 analysis items, initially the apparatus is set to that number of analysis items which can be analyzed at one time and the analysis is effected. Subsequently, the apparatus is set to the remaining analysis items and the analysis is effected again. As a result, the conventional apparatus has the drawback that the operations of setting the reagent and of setting the analysis condition for each analysis item become more complex and that there is a risk of erroneous operation being induced.

SUMMARY OF THE INVENTION

An object of the invention, therefore, is to provide an automatic analyzing apparatus which can reliably set the analysis items by means of a simple operation.

A feature of the invention is the provision of an automatic analyzing apparatus comprising means for discriminating kinds of reagent, a memory for storing analysis conditions for all of the analysis items such as an amount of sample to be injected, amount of reagent to be injected, photometric wave length, or the like, and a control device for controlling said means in response to the analysis condition read out from said memory.

Further objects and features of the invention will be fully understood from the following detailed description with reference to the accompanying drawings, wherein:

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BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a perspective view of one embodiment of an automatic analyzing apparatus according to the invention:

FIG. 2 is a diagrammatic view showing an arrangement of essential parts of the automatic analyzing apparatus shown in FIG. 1;

FIG. 3 is a perspective view of a reagent vessel used in the automatic analyzing apparatus shown in FIG. 1;

FIG. 4 is a perspective view of another embodiment of an automatic analyzing apparatus according to the invention;

FIG. 5 is a cross-sectional view of a reagent vessel holder set on a mounting member of the automatic analyzing apparatus shown in FIG. 4;

FIG. 6 is a perspective view of the reagent vessel holder shown in FIG. 5;

FIGS. 7A, 7B and 7C are diagrammatic views illustrating three discriminative examples of the reagent vessel holder shown in FIG. 6; and

FIG. 8 is a diagrammatic view showing a control circuit of an automatic analyzing apparatus shown in FIG. 4.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

FIG. 1 shows one embodiment of an automatic analyzing apparatus according to the invention. On the apparatus main body 1 are rotatably mounted a sampler 2 and reaction disc 3, these sampler and reaction disc being intermittently rotatable with a constant period. A plurality of sample cups 4 containing collected serum, urine or the like are arranged along the same circle having a center at the rotary axis of the sampler 2 and equidistantly separated one from the other. A plurality of cuvettes 5 are held by the reaction disc 3 and arranged along the same circle having a center at the rotary axis of the reaction disc 3 and equidistantly spaced apart from each other. The apparatus main body 1 is also provided with a compartment 6 formed in the apparatus main body 1 and detachably enclosing therein a plurality of reagent vessels 7 containing given reagents necessary for desired analysis items and with a detector 31 as shown in FIG. 2 and for discriminating the reagent vessels 7 one from the other. The apparatus main body 1 is provided with a probe 8 connected to a syringe 30 as shown in FIG. 2. The probe 8 functions to suck in, at a given sucking position, the sample in each sample cup 4 held by the sampler 2 and inject a given amount of the sample thus sucked together with the reagent in the given reagent vessel 7 set in the reagent vessel mounting member 6 to each reaction cuvette 5 held by the reaction disc 3. The reaction disc 3 is provided at its periphery with a probe 9 connected to a syringe (not shown). The probe 9 functions to inject a given amount of reagent in another given reagent vessel 7 set in the compartment 6 as a second reagent to each reaction cuvette 5 located at a position which is taken by each reaction cuvette 5 after the lapse of a given time from the exhaust position of the probe 8. In this way, the sample and second reagent are injected into the reaction cuvette 5 to prepare a test liquid. Then, during a given time, the reaction cuvette 5 is transferred to a position where the test liquid is subjected to the colorimetric measurement with the aid of a filter 21 which allows to pass a light having a given wave length there-through in response to the analysis item. The measured

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value and concentration conversion factor in response to the analysis item cause the desired analysis to be displayed by an output printer 10. In addition, the apparatus main body 1 is provided with an input keyboard 11 for causing a memory 36 to store beforehand analysis conditions such as an amount of sample to be injected, amount of reagent to be injected, photometric wave length, or the like. The apparatus main body 1 is also provided with a monitor cathode ray tube 12 for displaying the input information, analysis data or the like delivered from the input keyboard 11.

As shown in FIG. 2, provision is made of a control device 24 for controlling the analysis operation in response to the analysis condition for each analysis item. The control device 24 is constructed such that it functions to read out the analysis condition for the analysis item corresponding to the reagent discriminated by the memory 36 and control the analyzing operation of the desired analysis item.

FIG. 2 shows essential parts of the automatic analyzing apparatus shown in FIG. 1. Same reference numerals designate the same parts throughout FIGS. 1 and 2. To the center shaft of the sampler 2 is secured an output shaft of a pulse motor 15. The pulse motor 15 functions to intermittently rotate the sampler 2 with a constant period. The reaction disc 3 is provided at its periphery with a gear threadedly engaged with a gear secured to an output shaft of a pulse motor 16. The reaction disc 3 is so constructed that it is rotated intermittently with a given period in synchronism with the rotation of the sampler 2.

In the present embodiment, the reaction disc 3 is provided at its center portion with an opening in which is fitted a photometric device for effecting colorimetric measurement of the test liquid prepared in the reaction cuvette 5. The photometric device is constructed such that a light ray emitted from a polychromatic light source 17 arranged on the rotational center axis of the reaction disc 3 is incident on a lens 18 to obtain a parallel light flux which is then incident through a prism 19 on the reaction cuvette 5 arranged at a given photometric position of the reaction disc 3, that the light transmitted through the reaction cuvette 5 and the test liquid contained therein is received by a light receiver 20, and that in the photometric light passage between the light source 17 and the light receiver 20 is selectively inserted a filter 21 that functions to transmit therethrough a light having a given wave length in response to the analysis item. In the present embodiment, the filter 21 functions to transmit lights used for various analysis items and having different wavelengths and is held on the same circle having a center at the center axis of a filter holding member 22. The filter holding member 22 is rotatably supported at its center axis and driven by a pulse motor 23 connected to the center axis of the filter holding member 22 and inserted into the photometric passage. The pulse motor 23 is driven through a driver 25 from the control device 24. The output from the light receiver 20 is supplied through an amplifier 24, logarithmic amplifier 27 and analog-digital converter 28 to the control device 24.

The probe 8 is connected through a change-over valve 29 to the syringe 30 and to that reagent vessel 7 which contains a reagent used as a diluent and which is set in the compartment 6. The reagent vessel 7 set in the reagent vessel mounting member 6 is discriminated by a detector 31 arranged in opposition to the reagent vessel 7. The discrimination signal delivered from the detector

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31 is supplied through an interface 32 to the control device 24.

In the present embodiment, to each reagent vessel 7 used for various analysis items is attached a label 33 as shown in FIG. 3. The label 33 is provided thereon with code marks corresponding to the kinds of reagents contained in the reagent vessel 7 and to analysis item, for example, "GOT". The detector 31 functions to photo-electrically read out these code marks.

In FIG. 2, the syringe 30 is driven by a pulse motor 34 so as to effect its injection operation and control the amount of sample to be injected and amount of diluent to be injected in response to the analysis item. The driving control operation of the pulse motor 34 is effected through a driver 35 from the control device 24.

Similar to the probe 8, the probe 9 for injecting the second reagent to the reaction cuvette 5 is connected through the change-over valve 29 to the syringe 30 and to another given reagent vessel 7 set in the compartment 6. The amount of sample and second reagent to be injected by the syringe 30 is also controlled in response to the analysis item by means of the control device 24. Similarly, the reagent vessel 7 connected to the probe 9 is also discriminated by the detector 31 and the discrimination signal thus obtained is supplied to the control device 24.

A memory 36 functions to store the analysis items of all of the analyzable items delivered from an input keyboard 11 and supply the analysis condition of a given analysis item to the control device 24. The control device 24 functions to read out the analysis condition for the analysis items which have been stored in the memory 36 and corresponding to the reagent discriminated by the detector 31 and control the operation of the above described members such as the pulse motors 23, 34 or the like on the basis of the analysis condition and select the given concentration conversion factor so as to control the desired analysis item.

As stated hereinbefore, the present embodiment renders it possible to reliably set the desired analysis item by the simple operation of setting the reagent vessel 7 containing the given reagent necessary for the desired analysis item in the reagent vessel mounting member 6.

FIG. 4 shows another embodiment of an automatic analyzing apparatus according to the invention. In the present embodiment, provision is made of a reagent vessel holder holding a plurality of reagent vessels therein. An apparatus main body 41 is provided at its upper surface with a sampler 42 operative to intermittently transfer along a given passage a plurality of sample cups 42' containing various kinds of collected samples. The samples to be transferred in succession by the sampler 42 are injected in succession to a plurality of reaction cuvettes 43' held by a reaction disc 43 by a given amount by means of a sample syringe (not shown). The reaction cuvettes 43' are arranged along the same circle having a center at the rotary axis of the reaction disc 43 operative to be intermittently rotated with a constant period by means of a pulse motor (not shown). To each reaction cuvette 43' arranged on the reaction disc 43 and received the sample is injected a given amount of reagent in response to the analysis item by means of a probe 44 which constitutes a reagent syringe at a given position. As a result, a test liquid is formed in each cuvette 43'.

In the present invention, analysis on a plurality of given items is effected at one time by means of one analyzing operation. For this purpose, a plurality of

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reagent vessel 48 for containing reagents necessary for the analysis on the plurality of given items are held by a reagent vessel holder 45. The reagent vessel holder 45 is detachably mounted on a guide plate 57 secured to a base plate 54.

The test liquid prepared from the sample and reagent and contained in the reaction cuvette 43' is transferred to a given photometric position where the light which has passed through the filter for transmitting the light having a given wave length in response to the analysis item passes through the test liquid to effect the colorimetric measurement. Alternatively, the test liquid in the reaction cuvette 43' is sucked into a flow cell (not shown) on which is incident the light which has passed through the above mentioned filter to effect the colorimetric measurement. The quantitative analysis is effected by the value thus measured and the concentration conversion factor in response to the analysis item. The apparatus main body 41 is provided at its upper surface with a cover 46 operative to be opened and closed for the purpose of maintaining the reaction temperature of the test liquid substantially constant.

FIG. 5 shows in section the base plate 54 and reagent vessel holder 45 set thereon of the automatic analyzing apparatus shown in FIG. 4. FIG. 6 shows the reagent vessel holder 45 in itself. The reagent vessel holder 45 is composed of a turntable 47 which encloses therein a plurality of reagent vessels 48 arranged along the same circle having a center at the rotary axis of the reagent vessel holder 45 in a given order. The reagent vessels 48 contain reagents necessary for analysis on a plurality of given items. The turntable 47 is rotatably journaled through a bearing 49 by a holding member 50. The turntable 47 is provided at its center portion with a bore 51 adapted to insert therein a driving shaft 56 connected to the output shaft of a motor 55. Into a portion of the bore 51 is projected a pin 52. The turntable 47 is provided at its side wall with discrimination holes 53 for discriminating the analysis items which can be analyzed by the reagents in the reagent vessel 48 held by the turntable 47.

The reagent vessel holder 45 is mounted on the base plate 54 to which is secured the motor 55 and guide plate 57. To the output shaft of the motor 55 is secured the driving shaft 56 inserted into the bore 51 provided in the reagent vessel holder 45 and provided along its lengthwise direction with a slit slidably engageable with the pin 52. The guide plate 57 is secured to the upper surface of the base plate 54 and adapted to be engaged with the inner surface of the holding member 50 of the reagent vessel holder 45. A detector 58 is composed of photocouplers mounted on a supporting member 58' secured to the base plate 54 and cooperative with the discrimination holes 53 provided in the reagent vessel holder 45.

In the case of setting the reagent vessel holder 45 on the base plate 54, the holding member 50 is brought into engagement with the guide member 57 secured to the base plate 54 and the pin 52 projected into the bore 51 engages with the slit provided in the driving shaft 56. If the motor 55 is energized to rotate the turntable 47 by a given distance, it is possible to move the reagent vessel 48 containing the desired reagent to a given injection position of the probe 44 for constituting the reagent syringe as shown in FIG. 4.

In the present embodiment, the detector 58 is composed of three photocouplers. The lowest photocoupler is used to deliver an original point signal for indexing

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the reagent vessel holder 47 corresponding to organic ability to be examined. The original point signal thus delivered from the lowest photocoupler causes to move the desired reagent vessel 48 arranged in the given order to a given injection position. The other two photocouplers function to discriminate the reagent vessels 48 one from the other.

As shown in FIGS. 7A, 7B and 7C, the three discrimination holes 53 provided in the reagent vessel holder 45 are used such that the lowest hole shown by a full line in FIG. 7A can deliver the original point signal and that a combination of the other two upper holes can discriminate three kinds of reagent vessels 48 one from the other as shown by full line and dotted lines holes in FIGS. 7A, 7B and 7C.

FIG. 8 shows a control circuit for the automatic analyzing apparatus shown in FIG. 4. As described above, the detector 58 functions to discriminate the reagent vessel holder 45 set on the base plate 54 and deliver the discrimination signal. The discrimination signal is supplied through an interface 59 to a control device 60. In a memory 61 have beforehand been stored the analysis condition such as the amount of sample to be injected, amount of reagent to be injected, photometric wave length, concentration conversion factor or the like for each of all of the analysis items (in general more than 30 items) by means of a well known means. The control device 60 functions to read out from the memory 61 each analysis condition of the plurality of given analysis items (in general 6 to 12 items) which can be analyzed by the reagent vessel 48 set in the discriminated reagent vessel holder 45 on the basis of the discriminating signal from the detector 58. Based on the analysis condition read out from the memory 61, the operation of each member of the sample syringe, reagent syringe, rotation of the reagent vessel holder 45, selection of the optical filter or the like is controlled through the interface 62 and each driver 63 and a given concentration conversion factor is selected to deliver the desired analysis data as an output.

As stated hereinbefore, the automatic analyzing apparatus according to the invention is capable of precisely setting a plurality of desired analysis items by a simple operation of setting onto a mounting member a reagent vessel holder for holding a plurality of reagent vessels containing given reagents necessary for a plurality of desired analysis item. As a result, the invention can prepare a desired reagent vessel holder in response to a plurality of analysis items necessary for examining hepatic ability, kidney ability or the like.

What is claimed is:

1. An automatic analyzing apparatus comprising:
 - means for containing a plurality of samples to be analyzed in a plurality of containers;
 - means for delivering each of the samples into respective reaction vessels;
 - a plurality of reagent vessels containing plural kinds of reagents for effecting a plurality of analysis items;
 - a reagent vessel holder comprising a turntable for holding said plurality of reagent vessels along a circumference thereof;
 - a delivery device for delivering into the reaction vessels a reagent contained in a reagent vessel which is indexed at a delivery position;
 - means for rotating said reagent vessel holder in such a manner that the reagent vessels arranged in the

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reagent vessel holder are passed through said delivery position;

first indicia means provided in said reagent vessel holder for denoting an original point of the reagent vessel holder with respect to said delivery position; 5
 second indicia means provided in said reagent vessel holder for discriminating the kind of the reagents;
 means for detecting said first and second indicia means formed in said reagent vessel holder to generate an original point signal and a command signal 10
 for discriminating the kind of the reagent, respectively;
 means for storing a plurality of analysis conditions for all analysis items to be effected by the apparatus, said analysis conditions including an amount of 15
 sample to be delivered into the reaction vessel, an amount of reagent to be delivered into the reaction vessel and photometric wavelength;
 means for selecting in response to said command signal one of said plurality of the analysis conditions stored in said storing means, the selected analysis condition being related to an analysis item using the relevant reagent denoted by said command signal; and 20
 means for controlling said rotating means under the control of said original point signal and for controlling said delivering means in response to the analysis condition read out from said storing means. 25

2. The apparatus according to claim 1, wherein said reagent vessel holder is detachably engaged with the 30
 said rotating means.

3. The apparatus according to claim 2, wherein said rotating means comprises a base plate, a guide plate secured to upper surface of the base plate and a motor secured to a lower surface of the base plate, and said 35
 reagent vessel holder further comprises a holding member detachably engageable with said guide plate, a bearing arranged between the turntable and holding member and a post member provided in said turntable detachably coupled with said motor. 40

4. The apparatus according to claim 1, wherein said first and second indicia means are formed by indicia discrimination holes provided in the reagent vessel holder.

5. The apparatus according to claim 4, wherein: said 45
 detecting means comprises a detector composed of photocouplers mounted in a supporting member secured to an apparatus base plate and cooperative with said indicia discrimination holes provided in a reagent vessel holder so as to photoelectrically read out a reagent vessel corresponding to organic ability to be examined and also read out a plurality of reagent vessels held by a reagent vessel holder. 50

6. The apparatus according to claim 1, wherein: said 55
 controlling means is connected through an interface to said detecting means and through said storing means to an input keyboard, said controlling means being connected through drivers to pulse motors for driving a filter inserted into a photometric passage and for driv-

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ing a syringe for delivering reagent and sample into a reaction cuvette.

7. An automatic analyzing apparatus comprising:
 means for containing a plurality of samples to be analyzed in a plurality of containers;
 means for delivering each of the samples into respective reaction vessels;
 a plurality of reagent vessels containing plural kinds of reagents for effecting a plurality of analysis items;
 reagent vessel holder means for holding said plurality of reagent vessels;
 a delivery device for delivering into the reaction vessels a reagent contained in a reagent vessel which is indexed at a delivery position;
 means for moving said reagent vessel holder means in such a manner that the reagent vessels arranged in the reagent vessel holder means are moved to said delivery position;
 first indicia means associated with said reagent vessel holder means for denoting the indexing position of said reagent vessel holder means with respect to said delivery position;
 second indicia means associated with said reagent vessel holder means for distinguishing as to the kind of the reagents;
 means for detecting said first and second indicia means associated with said reagent vessel holder means to generate an indexing signal and a command signal for distinguishing the kind of the reagent;
 means for storing a plurality of analysis conditions for all analysis items to be effected by the apparatus;
 means for selecting in response to said command signal one of said plurality of the analysis conditions stored in said storing means, the selected analysis condition being related to an analysis item using the relevant reagent denoted by said command signal; and
 means for controlling said moving means under the control of said indexing signal and for controlling said delivering means and said delivery device in response to the analysis condition read out from said storing means;
 whereby said detecting means operates to generate a command signal which is responded to by said selecting means which selects an analysis condition from said storing means such that an analysis is performed automatically.
 8. The apparatus of claim 7 wherein said primary indicia means are utilized for determining the analysis items which can be analyzed by a reagent in said reagent vessel.
 9. The apparatus of claim 7 wherein said analysis conditions include an amount of sample to be delivered into said reaction vessel and an amount of reagent to be delivered into said reaction vessel.
 * * * * *

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EXHIBIT

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US005439645A

United States Patent [19]
Saralegui et al.

[11] **Patent Number:** **5,439,645**
[45] **Date of Patent:** **Aug. 8, 1995**

- [54] **APPARATUS FOR AUTOMATICALLY, SELECTIVELY HANDLING MULTIPLE, RANDOMLY ASSOCIATED HEMATOLOGICAL SAMPLES**
- [75] Inventors: **Francisco J. Saralegui**, Miami; **Alex W. Schlunkmann**, Plantation, both of Fla.
- [73] Assignee: **Coulter Corporation**, Miami, Fla.
- [21] Appl. No.: **9,190**
- [22] Filed: **Jan. 25, 1993**
- [51] Int. Cl.⁶ **B01F 11/00; G01N 35/02**
- [52] U.S. Cl. **422/64; 366/128; 366/218; 422/63; 422/67**
- [58] Field of Search **422/63, 64, 65, 67; 435/312, 316; 366/128, 218**

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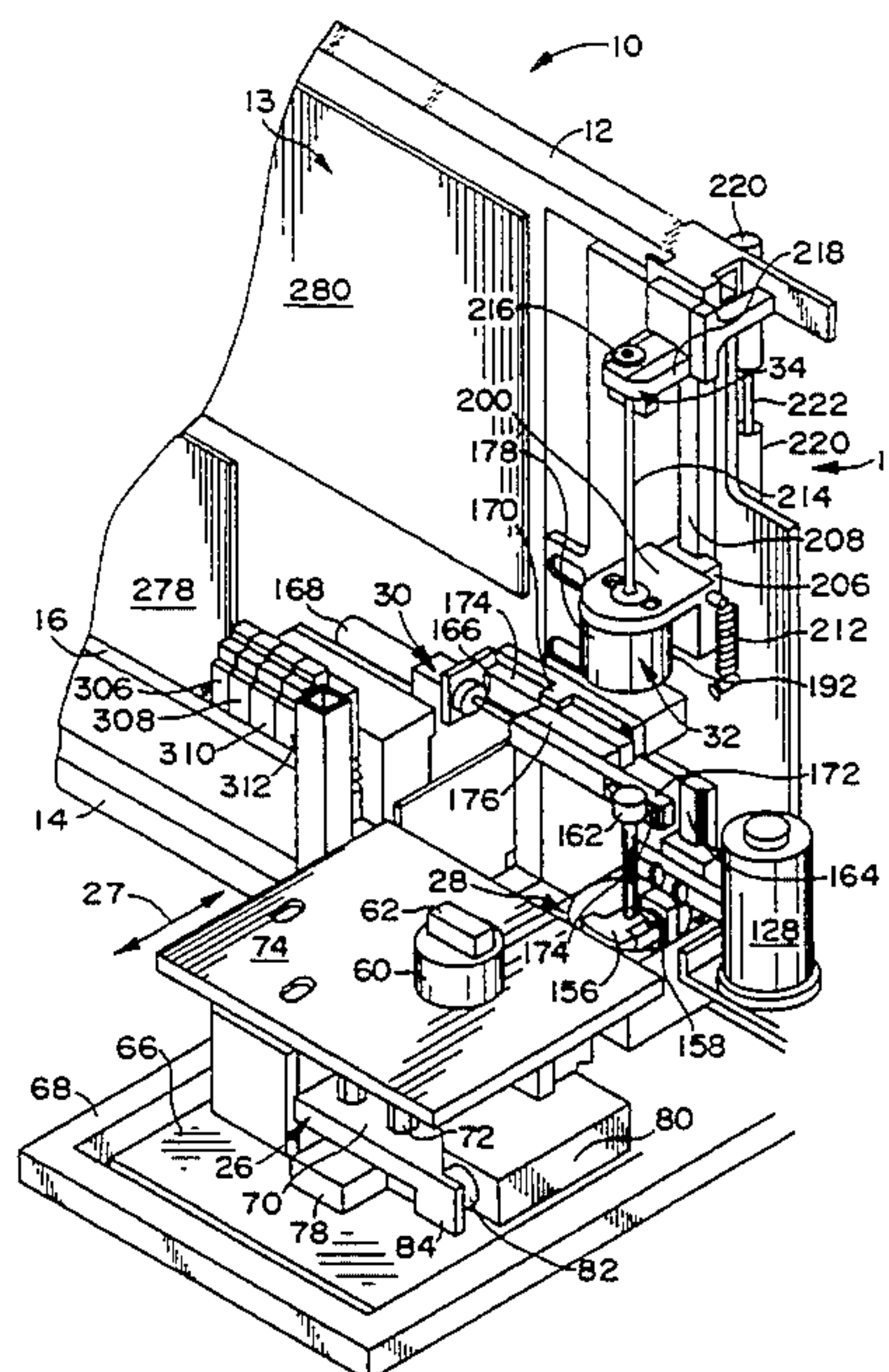
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Primary Examiner—Robert J. Warden
Assistant Examiner—Robert Carpenter
Attorney, Agent, or Firm—Carl Fissell, Jr.; John T. Winburn; Mitchell E. Alter

[57] **ABSTRACT**

An apparatus which provides an automatic, signal controlled, sample mixing/resuspending, aspiration and delivery system; wherein a demountable, rotatable carousel temporarily holds a multiplicity of sample containers. Electro-mechanical means, including optical sensors, is programmed to automatically move the carousel to a pre-selected position. A self centering vortexer/mixer lifts a selected sample container from the carousel, sealingly engages the sample container within a sample container support and thereafter orbitally mixes and resuspends the sample container contents. An aspiration probe enters the sample container through the support to receive the sample, which is forced out of the sample container by means of compressed air introduced into the sample container via the sample container support. The sample is delivered to an operably associated flow cytometer via the aspiration probe, after which the probe is washed and readied for the next cycle of operation.

13 Claims, 8 Drawing Sheets



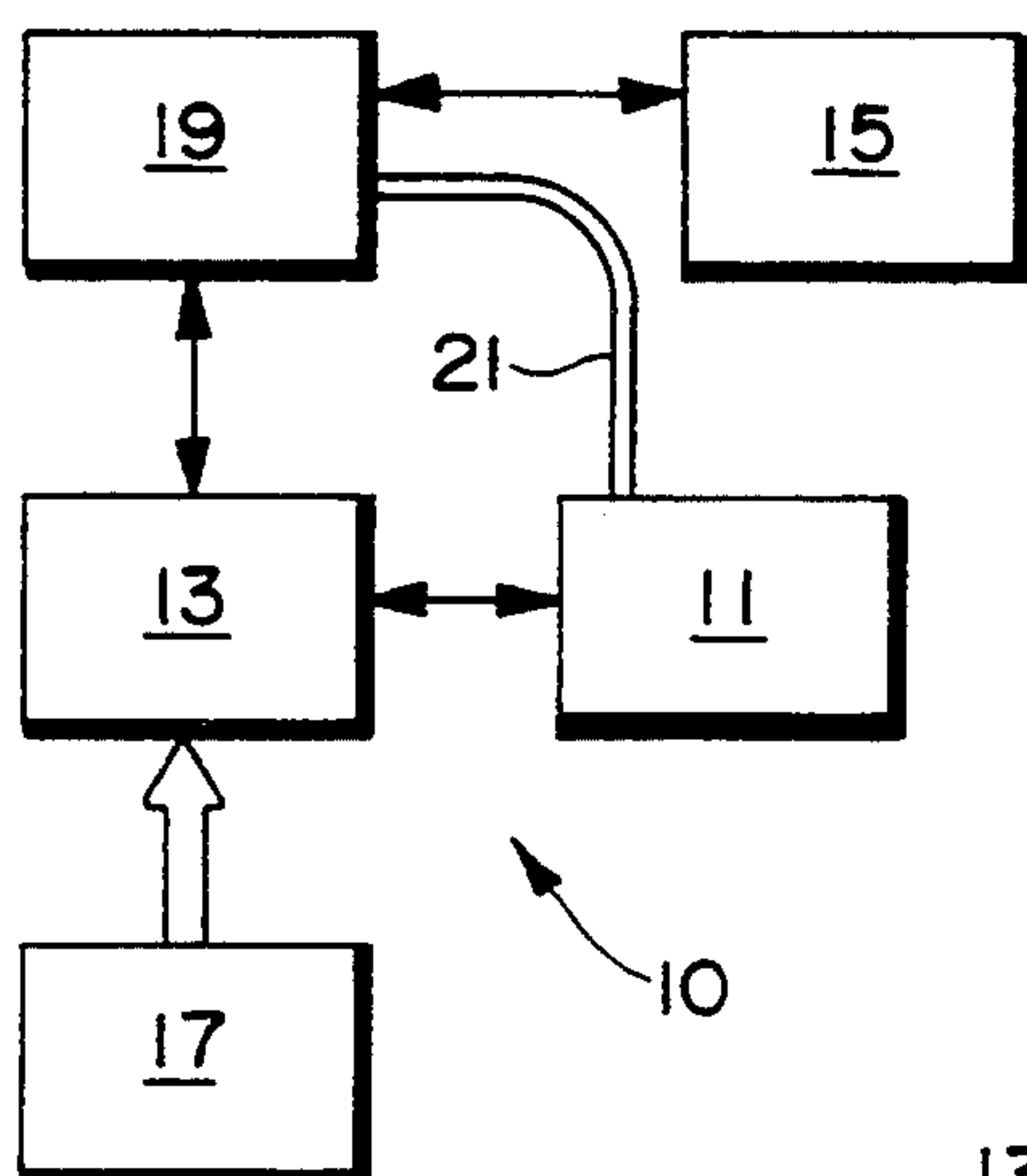


FIG. 1

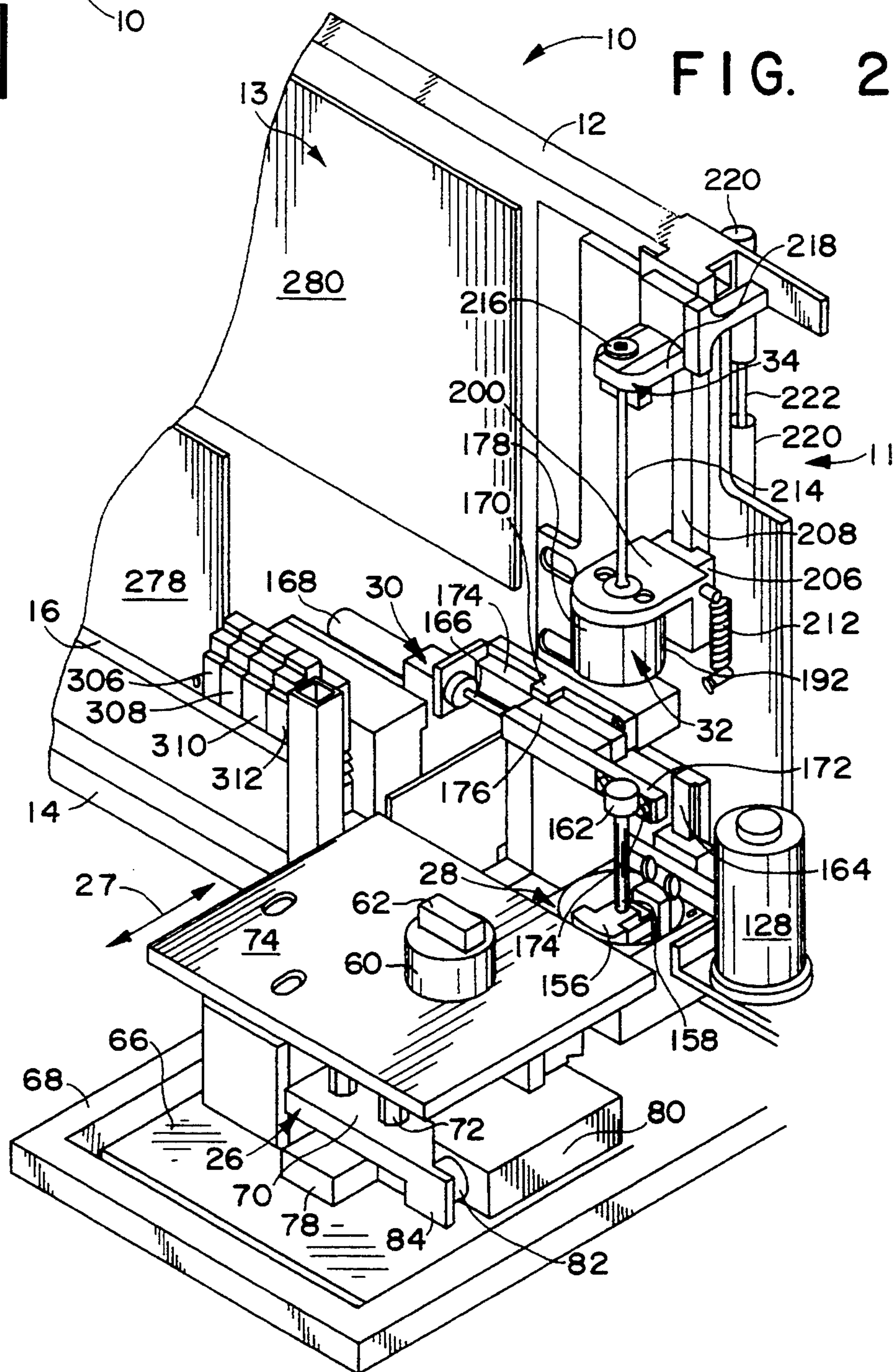


FIG. 2



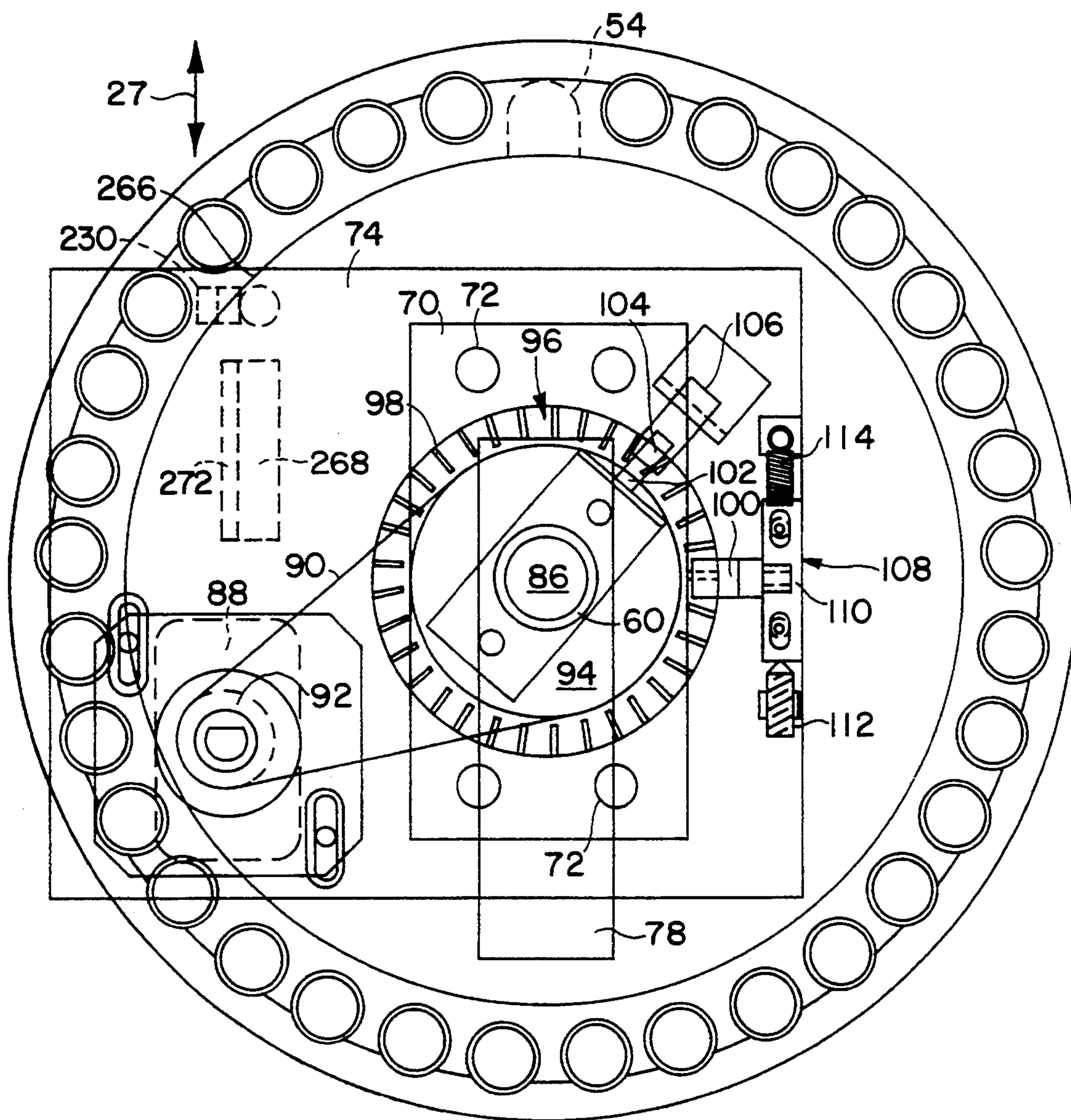


FIG. 5

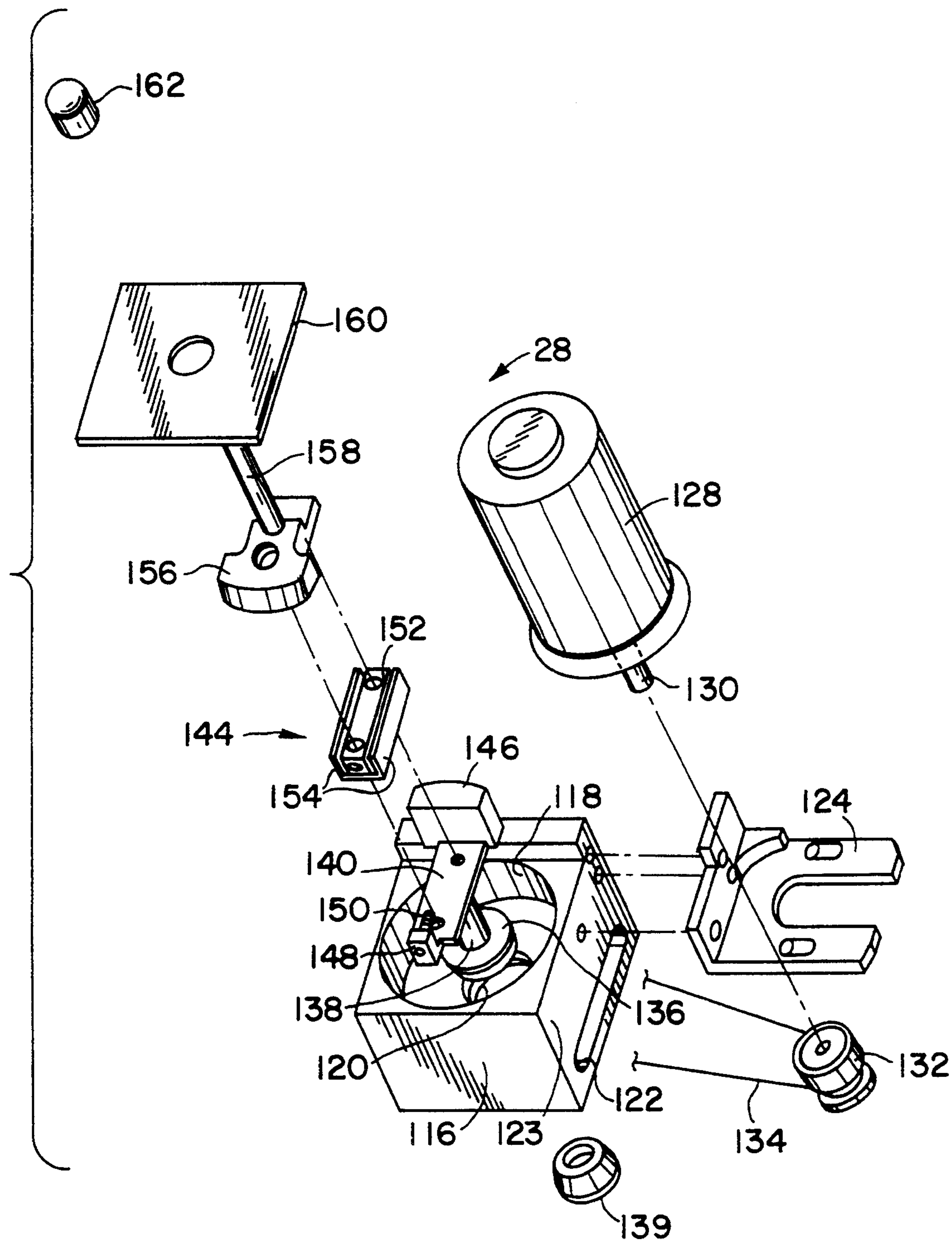


FIG. 6

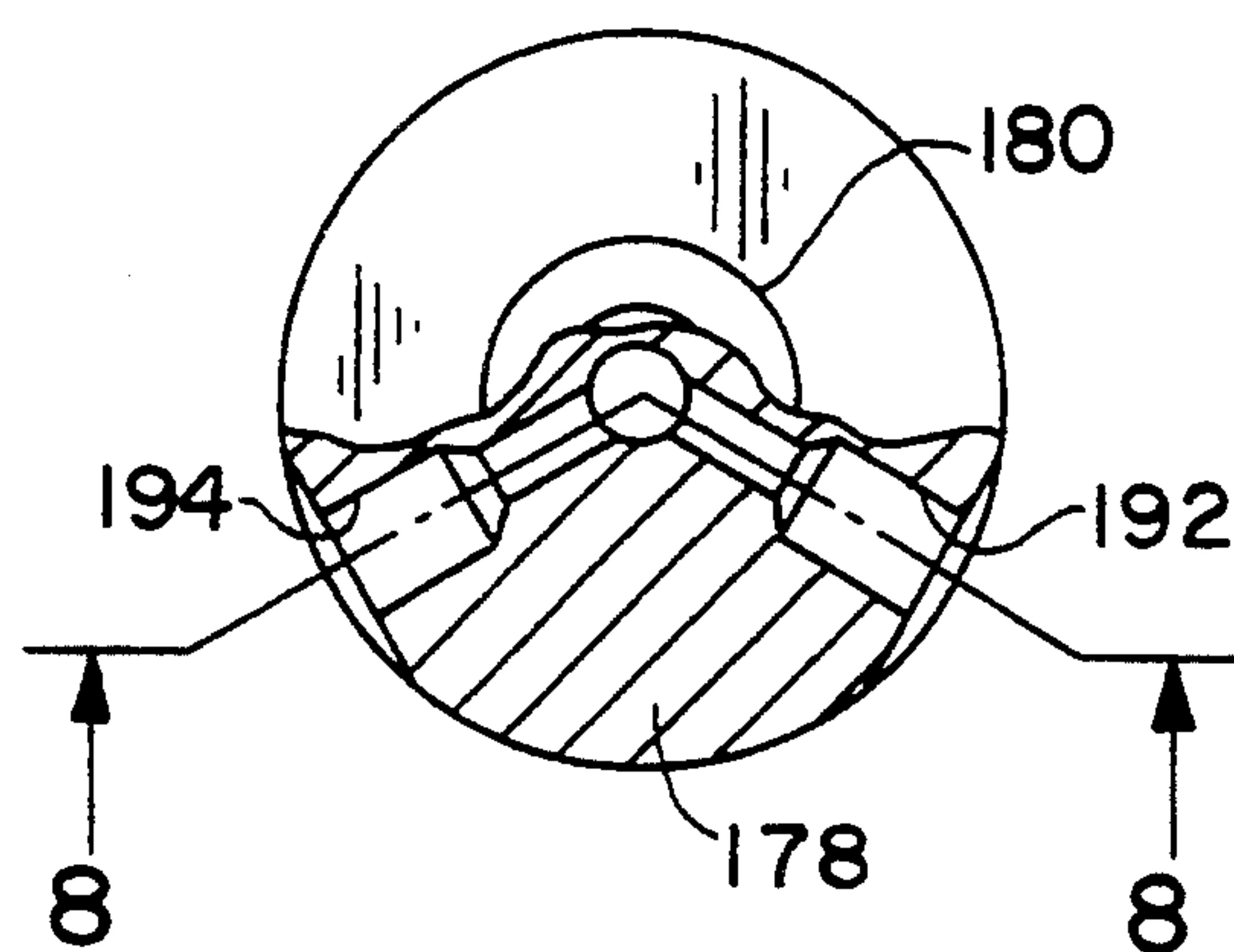


FIG. 7

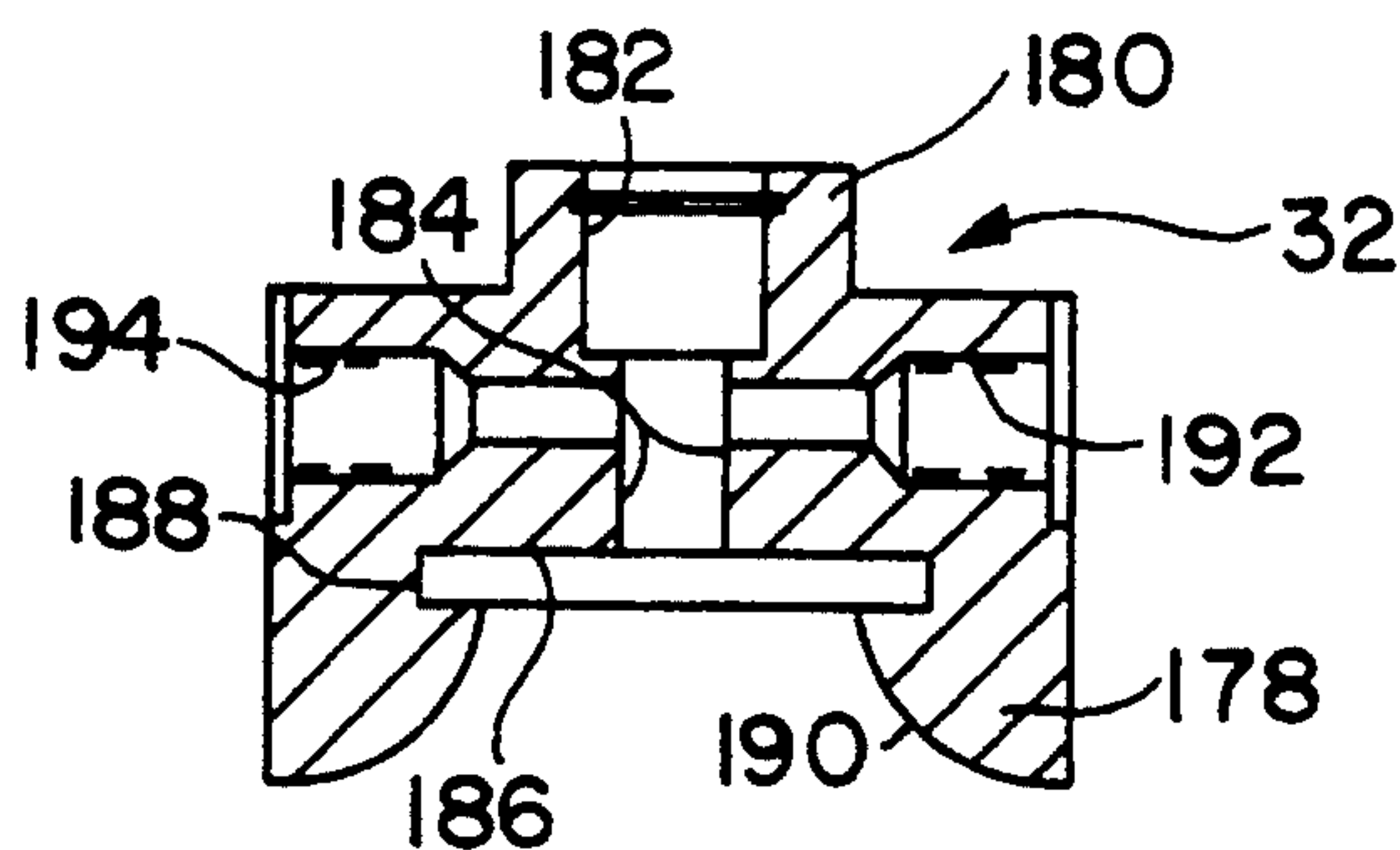


FIG. 8

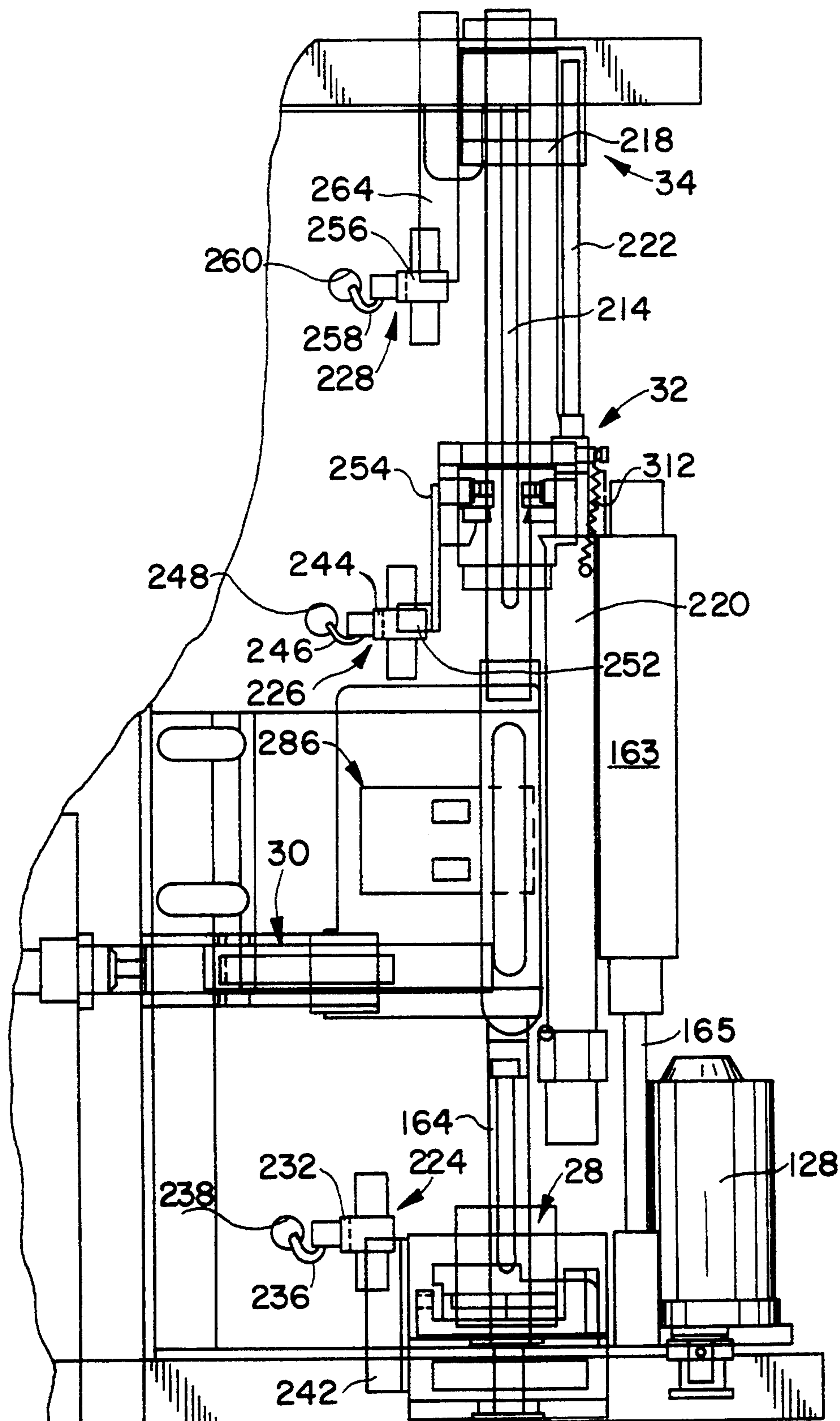
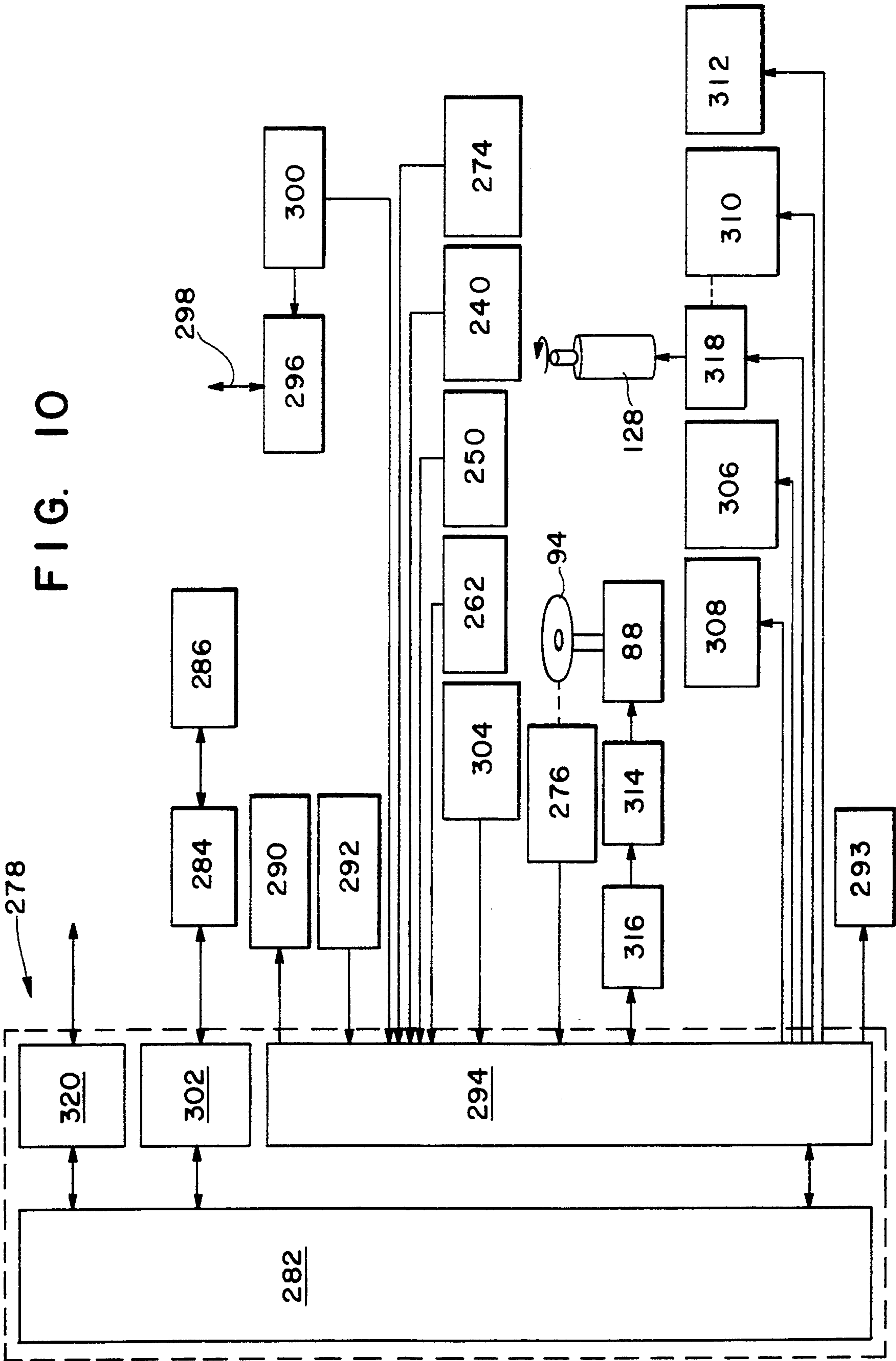


FIG. 9



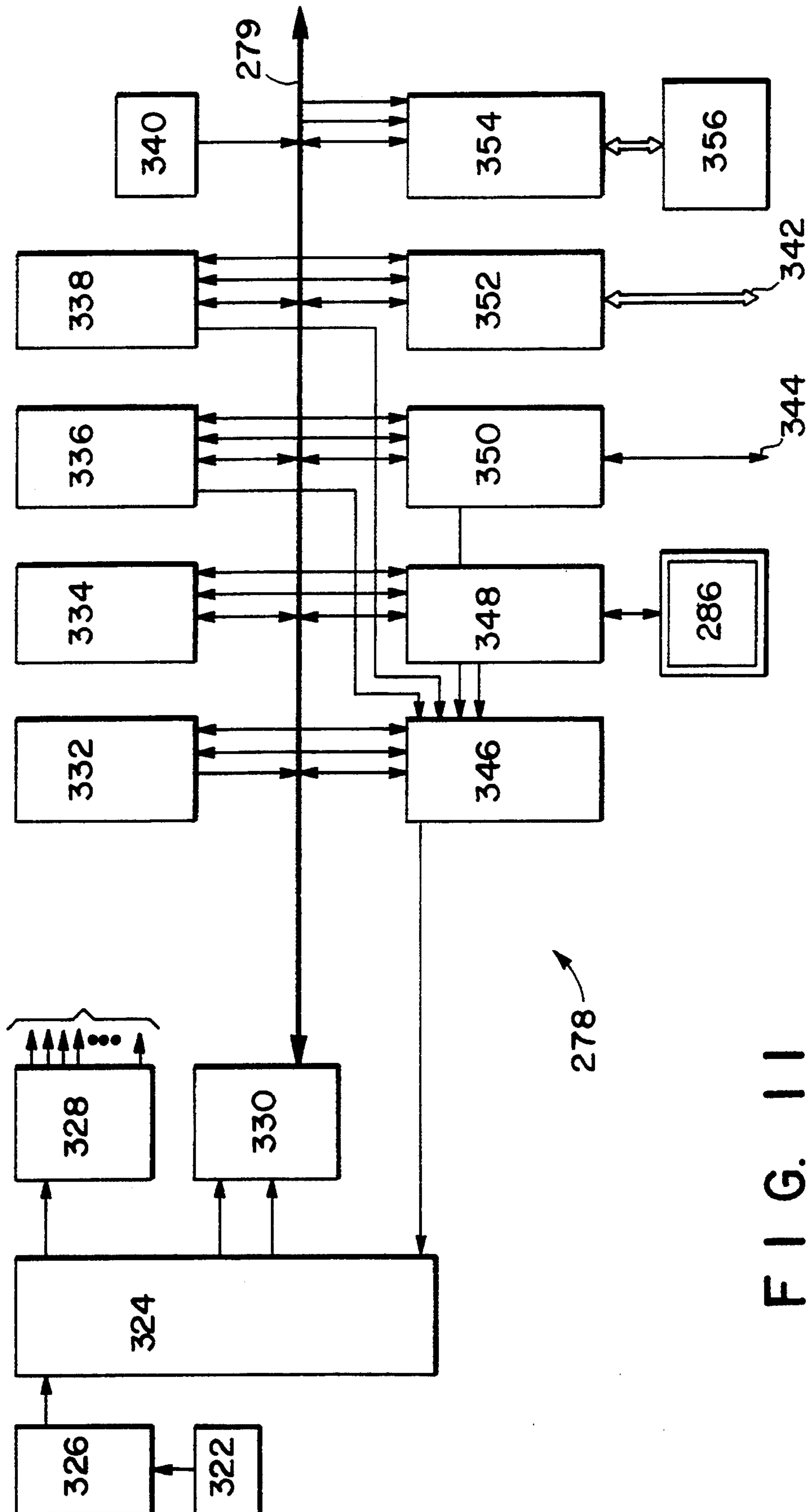


FIG. 111

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APPARATUS FOR AUTOMATICALLY, SELECTIVELY HANDLING MULTIPLE, RANDOMLY ASSOCIATED HEMATOLOGICAL SAMPLES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to apparatus for automatically handling a multiplicity of randomly associated hematological samples, and, more particularly, concerns apparatus for automatically mixing, resuspending and aspirating pre-prepped blood samples and transferring such resuspended samples to other operably associated cytometric apparatus.

2. Description of the Prior Art

U.S. Pat. No. 4,845,025, entitled "Biological Sample Mixing Apparatus and Method" relates to automatic biological sample mixing apparatus for use in flow cytometric apparatus, wherein a sample container is secured loosely at its top by means of a multi-reagent dispensing head; and the container is mounted at its bottom on a resilient support disposed on an elliptically rotatable member, to cause reagents introduced into the container to be thoroughly mixed with a sample in the container in a fast, efficient, gentle, and accurately repeatable manner.

A recent improvement upon the COULTER® Q-Prep and also protected by U.S. Pat. No. 4,845,025 is the COULTER® Multi-Q-Prep which consists of a 32-tube capacity removable carousel, sensor and sequencing devices, a reagent delivery system, mixer and timing devices. An indexing base moves the carousel in counterclockwise direction to a home position and during sequential sample processing. A tube detector/lifter senses the presence of a sample containing tube in the carousel at mixing position and lifts it up into the dispensing head. A retractable dispensing head adds precise amounts of reagents to the sample. A vortexer/mixer mixes reagents into the sample.

Such prior art, while capable of efficiently and accurately mixing various reagents with the sample, requires that the mixed or pre-prepped sample be removed manually from the mixing apparatus (the COULTER Q-Prep or Multi-Q-Prep) and also the tube and transferred manually to a sample analyzer, for example, a flow cytometer such as the COULTER® EPICS® Profile or XL. Both apparatuses utilize pressure syringes to provide fluid movement. The Q-Prep apparatus does not use bar codes or a bar code reader, inasmuch as it operates on a single, operator manually delivered sample container, one at a time. The Multi-Q-Prep carousel has its 32 tube positions bar coded for sample I.D. Also, these two patented apparatuses rotate in one direction only; and one utilizes a cam and lever operated drive and the other has a notched belt for container rotation and reagent mixing. Vacuum devices and pumps, as used in the apparatus, are relatively expensive, require continuous care and maintenance and the cost is sometimes considerable to the point where it might become prohibitive for small laboratories or medical offices.

SUMMARY OF THE INVENTION

The present apparatus avoids the foregoing shortcomings by providing an automatic, motor driven, signal controlled, sample resuspending, aspiration and delivery system wherein a demountable, rotatable carousel is provided for temporarily holding a multiplicity

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of individual sample containers. Each sample container holds an individual sample and carries a bar code readable indicia for identifying the patient and for other data. The carousel also includes a discrete identifying bar code, as well as a series of individual sample container position identifying bar codes for indicating the location of each sample container disposed on the carousel.

Means are provided for moving the carousel to a pre-selected position and thereafter for removing a single sample container from the carousel and moving the container into sealing contact with an aspirating head probe, while orbitally rotating the selected sample container so as to mix and resuspend the container contents. Air pressure providing means, secured to the sample tube support head, forces air under pressure through the head into the selected sample container, forcing the container contents, or a portion thereof, out of the container through the sample aspirating probe and into means interconnecting the sample head with operably associated flow cytometry devices or equipment. Means for flushing and washing the sample aspirating probe also is incorporated in the sample tube support head.

The present apparatus also includes a high efficiency, high speed bar code reader, for reading the coded indicia on each sample container as well as for reading the carousel position code and the position code of each sample container on the carousel. Electronic control circuitry operably associated with this apparatus enables all operations to be performed in a fail-safe manner, such that tube jams, tube breakage or absence of tubes from the carousel can be noted, identified by position and action taken to avoid stoppage, breakdown or personnel injury. The present apparatus is completely enclosed by suitable closure members of metal or plastic, which members can be opened to provide operator access for loading, operation, maintenance, repair, cleaning and/or replacement of parts.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic block diagram of the present invention, as used with flow cytometric apparatus;

FIG. 2 is an isometric view of apparatus embodying the present invention;

FIG. 3 is a top plan view of the sample holding carousel used with this invention;

FIG. 4 is a sectional view along line 4—4 of FIG. 3;

FIG. 5 is an enlarged top plan view of the carousel drive train and operably associated position sensors, although portions of this figure are actually obscured from view by reason of mechanical overlays, the figure is nevertheless illustrated in full rather than dotted line to avoid unnecessary visual confusion;

FIG. 6 is an exploded view of the self centering lifter vortexer/mixer for the invention;

FIG. 7 is a sectional view of the sample tube support head;

FIG. 8 is a view along the line 8—8 of FIG. 7;

FIG. 9 is a front view of the apparatus of FIG. 2 illustrating various sensing devices used with the present invention; and

FIGS. 10 and 11 are functional block diagrams of the electrical and electronic control apparatus for the present invention.

DESCRIPTION OF AN EMBODIMENT OF THE INVENTION

Apparatus embodying the present invention, as illustrated in the drawings, is adapted to perform in a multi-functional capacity as a completely self-contained, modular unitary assemblage as an operational adjunct to an operably associated flow cytometer, such as the COULTER® XL, Profile or Elite. The present apparatus accomplishes a plurality of related functions, wherein separate structural assemblies are cooperatively combined and integrated into a single, modular, free-standing apparatus 10.

The apparatus 10 can be employed for hematologic sample handling used by a hematology laboratory, researchers and in hospitals. The operation of the apparatus is completely automatic and does not require an attendant technician to monitor its progress or its output results. A laboratory technician can load the apparatus with a sample carrying carousel, start the apparatus and walk away to perform some other laboratory or office function.

As seen in the block diagram of FIG. 1, the modular apparatus 10 comprises four major assemblies: a mechanical assembly 11, an electrical control assembly 13, a work station 15, and a software assembly 17. The assemblies 11-17 can be considered together to provide a Multi-Q-Prep Carousel Loader or MCL. A receptor assemblage 19 can include any flow cytometer, for example, a COULTER® Elite, Profile or the XL, depending on the particular requirements of the user researcher, laboratory or hospital.

As clearly seen in the isometric view of FIG. 2, the mechanical assembly 11 and the electrical control assembly 13 are structurally combined into the modular apparatus 10, which comprises a plurality of functionally integrated, mechanical and electrical subassemblies. The mechanical assembly includes a vertically disposed support member 12, which is secured at its base 14 to an L-shaped member 16.

The apparatus 10 includes among other things, a sample tube carrying carousel 20 (FIG. 3), having sample tubes 22 thereon. The sample tubes 22 can be standard test tubes of glass or plastic. Also included in the apparatus 10 is a disc-shaped carousel support 24 (shown in dotted outline in FIG. 3); a horizontal slide mechanism 26 (FIG. 2) for moving the carousel 20 and carousel support 24, back and forth in the direction of the two-headed arrow 27 (FIG. 2); a self-centering lifter vortexer/mixer 28, for resuspending the contents of the sample tubes 22, carried by the carousel 20; a pivoted, horizontal, sample tube rotating and steadying member 30; a sample tube support head 32; and a vertically movable sample aspirating member 34.

Located at various fixed positions on the apparatus 10, are a plurality of individual position locating and sensing devices, which will be described in detail with respect to FIG. 8. To the left of the mechanical assembly and operating mechanisms 11 just described are a plurality of electronic control components secured to the vertical support wall 12, making up the electrical control assembly 13 for controlling and directing the automatic operation of the MCL apparatus 10. The electrical control components are disposed on two printed circuit boards 278 and 280.

The operation of the MCL apparatus 10 is completely automatic and can be energized from an operably associated flow cytometer as part of the receptor assem-

blage 19 (FIG. 1) by way of interconnecting sample delivery tubes 21 and a software processor of the software assembly 17, which can be run on the flow cytometer 19.

The carousel 20 (FIG. 4), for supporting and transporting the sample tubes 22, is constructed as a shallow circular, rigid member 36, provided with a central, vertically projecting handle 38 and a series of thirty-three peripheral, fairly shallow, cylindrical pockets 40, to slidably receive an individual sample container tube 22. The tubes 22 project upwardly from the respective pockets 40 a sufficient distance to expose an individual bar code indicia label 42 secured around the surface of each tube 22. Each pocket 40 is identified by a position number 44, which is disposed on the upwardly exposed face of the carousel 20. A bar code label 46 also is applied to the rim portion 48 of the carousel 20, at each position corresponding to a pocket on the carousel.

The carousel 20 is demountably mountable onto the circular carousel support member 24 (dotted outline in FIG. 3), which is received within an inner, hollow, cup-shaped area 52 (FIG. 4) of the carousel 20. At a position corresponding to a so-called "home" position, the rim of the support member 24 is provided with an outwardly extending projection 54 (FIG. 3), engagable with and receivable within a matching notch 56 in the carousel 20. The support member 24 is attached to a stub shaft 60, having a horizontal integral rib 62 (FIGS. 2, 3) at its top. The rib 62 receives a matching rib-shaped slot 64 in the bottom center portion of the support member 24.

A base plate 66 (FIG. 2) is disposed on a forwardly extending portion 68 of the mechanical assembly 11 and provides a base for the horizontal slide mechanism 26. The slide mechanism 26 comprises an L-shaped support 70, provided with four pedestal members 72, which support plate 74. The support 70 is slidably mounted on an elongated guide member 78 and is adapted to be moved forwardly and rearwardly double headed arrow 27, by means of a double acting air cylinder 80, a piston shaft 82 of which is connected to a depending tang 84 on the vertical end of the support 70. A forward stop member (not shown), secured to the base member 14, acts to limit the inward travel of the slide mechanism 26.

FIG. 5, as earlier mentioned, is illustrated in full line rather than dotted line, since it is believed that dotted lines detailing one portion of the apparatus obscured by other portions of the apparatus would confuse rather than illuminate the subject matter. The specification clearly and succinctly locates and describes all portions of the apparatus, whether in plain sight or hidden from view. As seen in FIG. 5, the circular stub shaft 60 is secured at its lower end to a drive shaft 86 which extends vertically upwardly through the plate 74. An electric stepper-type drive motor 88 is supported beneath the plate 74. A drive belt 90 extends from a drive pulley 92 on the motor 88 to a driven pulley 94 on the drive shaft 86. An otherwise opaque disk 96 is provided with a plurality of radial, light-passing areas or stripes 98 and is secured to and rotatable with the drive shaft 86.

A sample tube 22 position sensor 100 is secured beneath the plate 74 and includes a yoke or U-shaped sensor which extends outwardly to straddle the perimeter of the disk 96 and permit the disk, when rotated, to interrupt light which is passed between a photodiode on one side thereof to a photoreceptor on the opposite side thereof (neither of which are shown in FIG. 5). A light

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interrupting member 102 is rotatable with the drive shaft 86 and projects between ends of a U-shaped carousel home position sensor 106, for purposes to be explained.

In order to ensure the accuracy of the centering alignment of each tube 22, an additional adjustment of the MCL apparatus 10 is provided by means of an adjustment member 108, FIG. 5. The tube position sensor 100 is mounted on a slidably movable member 110, slidably secured to the underside of the plate 74. A set screw 112 is provided to move the member 110 horizontally a slight distance against the tension of a spring 114. By adjusting the member 108, the tube position sensor 100 can be moved slightly, so as to position a tube 22 exactly centered under the sample tube support head 32, as well as precisely over the center of the LV mixer 28.

Between the carousel support slide mechanism 26 and the rear vertical wall 12 is the self-centering, lifter vortexer/mixer 28. The vortexer/mixer 28, as seen in detail in FIG. 6, comprises a rigid, cube-shaped member 116 having a large, central bore 118, concentric with a smaller bore 120, extending from the top surface of the cube 116 completely therethrough. One side of the cube 116 has an oval-shaped orifice 122 extending from side surface 123 into the central bore 118. An L-shaped bracket 124 is attached to the surface 123 and supports a drive motor 128 having a drive shaft 130 and a drive pulley 132. A drive belt 134 extends from the drive pulley 132 through the lozenge-shaped opening 122 and engages a driven pulley 136 which is mounted on the lower end of a vertically disposed shaft 138. The lower end of shaft 138 is rotatably mounted in a bearing member 139 secured into the bottom of the cube 116. The upper end of the shaft 138 carries a support 140, to which a ball slide 144 is secured. At one end of the support 140 is a small upstanding weight 146; and at the opposite end of the support 140 is a stop block 148, from which a small spring 150 projects toward the weight. The slide 144 has an inner portion 152 which is moveable horizontally between upstanding guide rails 154. A counter weight or imbalance member 156 has a vertically disposed shaft 158 centered thereon. The shaft 158 projects through a cover plate 160 and includes a small, slightly resilient, spherically hollowed knob or button 162 for non-damaging engagement with the bottom of the sample container tubes 22.

In the inactive position, without power being supplied to the drive motor 128, the spring 150, at one end of the ball slide 144, centers and aligns the vertical lifting/erecting shaft 158 with the drive shaft 138. In this aligned position, the spherically hollowed knob or button 162 can be accurately positioned beneath the bottom of a sample tube 22. When power is applied to the drive motor 128, the imbalance weight 156, as a result of the rotative force, causes the shaft 158 to move horizontally outwardly into a circular orbit, creating a vortexing/mixing action with respect to the contents of the tube 22. When the drive motor is turned off, the shaft 158 returns to the center position as a result of the absence of rotative torque; thus automatically self-centering the shafts 138 and 158.

With reference to FIG. 2, the self-centering lifter vortexer/mixer 28 is slidable vertically along a vertical guide 164 which is attached to the vertical wall 12 by means of a guide yoke (not shown) secured to the rear side of the cube-shaped member 116. Vertical movement of the lifter vortexer/mixer 28 is provided by means of the double acting air cylinder 163, the piston

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shaft 165 of which is connected to the rear portion (not shown) of the cube member 116. This movement of the vortexer/mixer 28 provides translational movement of the individual sample tubes 22, with respect to the tube carrying carousel 20.

Disposed at right angles to the vertical disposition of the lifter vortexer/mixer shaft 158 and at the level of the bottom of a tube 22, when in position, is the tube rotating and steadying member or finger 30. The finger 30 projects horizontally outwardly away from the shaft end 166 of an air cylinder 168. The finger member 30 is pivotally mounted at its center 170 and is attached at one end to the air cylinder shaft 166. The opposite, free end, 172 of the finger 30 is provided with a flexible, shock absorbent pad 174, of rubber or similar resilient material. A double acting air cylinder 176 is secured to the rear of the finger 30 for extending and retracting the finger 30 into and out of the path of movement of the lifter vortexer/mixer shaft 158, during each operation of the MCL apparatus 10.

The sample tube support head 32, as illustrated in FIGS. 2, 7 and 8, comprises a cylindrically shaped body 178 and integral concentric attachment member 180 (FIG. 8). A series of concentric bores 182, 184, and 186, extend downwardly within the body, with the outermost bore 186 having an undercut 188. The lowermost portion 190 of the body 178 is outwardly flared. The body 178 further includes outwardly extending, angularly offset, passageways 192 and 194, which open into the central bore 184 and provide means for mounting conduits (not shown). Fluid can be introduced into the passageway 192 to swirl downwardly into a sample tube 22. The passageway 194 is to be coupled to a source of vacuum, which is used to apply vacuum to the sample tube support head 32 to remove any residual fluid remaining after each operation.

The attachment member 180 of the support head 32 is disposed within a circular opening 198 in an L-shaped bracket 200, the rear portion of which is mounted to a slide block 206 (FIG. 2), which is slidable up and down along a guide rail 208, which is secured to the front surface of the wall 12. A coiled spring 212, one end of which is attached to the support head 32 and the opposite end of which is attached to the wall 12, biases the support head 32 upwardly.

The sample aspirating member 34 is employed for directing sample fluid under pressure from the sample tubes 22 into the flow cytometer 19 via the sample delivery tube 21. As shown in FIG. 2, depending from the aspirating member 34 is a probe 214, having a small opening at its lower end. The upper end of the probe 214 is flared outwardly to provide a retaining rim 216 for demountably supporting the probe 214 in a vertically slidable block 218, which acts as a support for the probe 214 and is arranged to be moved up and down along the guide rail 208. An air cylinder 220 has its plunger 222 secured to the slide block 218 for moving the aspirating probe 214 up and down, as called for during apparatus operation.

The MCL apparatus 10 employs a variety of electromechanical sensors which are used to indicate, monitor and cause the adjustment of the status of various of its portions. Not all of these sensors will be described in detail, either because they form part of a specific chip component, or because their operation is thought to be obvious from the general operational description. Four of the sensors are utilized to make the MCL apparatus 10 efficient, simple to operate, failsafe, and automatic

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with due regard to reliability and safety. As seen in FIG. 9, three of these four sensors (starting at the bottom of the drawing) include an LV mixer up/down detect sensor 224, a sample head detect sensor 226, and a sample probe detect sensor 228. The fourth sensor, a carousel in/out detector 230, will be described later in connection with FIG. 5. All four sensors are substantially identical in construction and operation; although each has an actuator which differs slightly from the others by reason of its separate function.

The LV mixer up/down detect sensor 224 includes a U-shaped sensor body 232, with a photooptical LED on one arm of the U and a photoreceptor on the opposite arm of the U (neither of which are shown in the figure). The sensor body 232 is secured to the support wall 12. Electrical lead wires 236 are arranged to pass from the sensor body 232 through a grommated aperture 238 in the wall 12 for interconnection to a LV up/down detect chip 240, shown in FIG. 10. An actuator member 242 for the LV up/down mixer sensor 232 comprises a flat, opaque strip of metal or similar material, which is secured to and moves with the LV/vortexer mixer 28. The actuator of each sensor interrupts the light passing from the LED of a sensor to its opposite positioned photoreceptor. An output signal thus produced is used to indicate and control the status, for example, of the LV mixer member 28 via the LV up/down detect chip 240.

The sample head detect sensor 226 includes a U-shaped sensor body 244 which is mounted to the wall 12. Its electrical lead wires 246 pass through a grommated aperture 248 and are interconnected to a head detect chip 250 on the printed circuit board. An actuator member 252 for the sample head detect sensor 226 comprises an opaque member integral with an elongated attachment member 254 which is secured to the side of the sample tube head support 32.

The sample probe detect sensor 228 has a sensor body 256 and its electrical leads 258 pass through a grommated opening 260 for connection to a sample probe detect chip 262. Probe actuator 264 is elongated and is attached to the vertically slidable block 218.

The fourth sensor in the group, the carousel in/out detect sensor 230, is located beneath and depends from the slidable carousel plate 74, as shown in FIG. 5. The sensor body 266 forms a gap between two opposing arms of its U-shape. Actuator 268 is secured to the base 66 (FIG. 2) and is L-shaped. An upstanding flange 272 of the actuator 268 is adapted to pass through the gap in the sensor 230 as the carousel 20 is moved in and out in the direction of the two-headed arrow 27. Electrical lead wires (not shown) connect the sensor 230 to the carousel in/out detect chip 274 (FIG. 10).

The operation of carousel sensor 230 is extremely important to the overall operation of the MCL apparatus 10. The sensor 230 indicates to the carousel in/out detect chip 274 the positional status of the carousel. It is essential to the MCL operation that the carousel be correctly positioned with respect to the lifter vortexer/mixer up/down apparatus 28, otherwise the lifter mixer 28 could be actuated prior to a sample carrying tube 22 being in position over the lifting/erecting shaft 158. Incorrect centering of the shaft 158 with respect to the tube 22 could cause the vertical lifting movement of the erecting shaft 158 to break or jam the sample tube, possibly contaminating the entire work area and causing a health hazard, or damage to the apparatus. The carousel in/out detect sensor 230 prevents these problems,

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The operation of this sensor is substantially identical to that of the other two sensors.

The MCL 10 has two (2) position sensing mechanisms 106 and 100 to determine the location of the carousel 20. One is utilized to place the carousel near the actual "home" position, the other to accurately position the carousel at any of 33 tube positions (including "home").

The stepper motor 88, previously referred to, controlled by the system hardware and software, turns the carousel. Positioning accuracy is approximately 0.15 thousands of an inch. By reading the state of the positioning sensors 106 and 100 and pulsing to the stepper motor 88, the MCL controller 282 can position the carousel to any desired location. The tube position detect sensor 100 and the home position detect sensor 106, are electrically connected to a tube position/home position detect chip 276 (FIG. 10). Both sensors 106 and 100 are shown in FIG. 5 and are substantially identical in construction and operation to that of the other sensors hereinbefore described.

The MCL apparatus 10 has a hinged cover (not shown) for fitting over at least the carousel 20 and the sample tubes 22 to inhibit aerosol from the open top tubes from entering the work area and also to prevent contaminants from falling into the tops of the tubes. The hinged cover activates a switch (not shown) which is coupled to the cover interlock detect chip 304 of the CPU controller 282, such that each time the cover is opened, the computer program in the CPU controller 282 causes the stepper drive motor 88 to rotate the carousel support 24 so that the projection 54 stops at a "load" position. The "load" position is 90° to the left of the "home" position in which the projection 54 is pointed toward the rear of the MCL apparatus 10, as illustrated by the two-headed arrow 27 in FIGS. 2, 3 and 5. When the carousel 20 is placed onto the support 24, which then is in the "load" position, the cover is closed and its switch, via the CPU controller, directs the stepper drive motor 88 to rotate the carousel 20 to the "home" position, with the projection 54 to the rear of the MCL apparatus 10. The two sensors 100 and 106 control the rotative positioning movement of the carousel 20, as now will be described.

The Home Sensor Mechanism 106 consists of a fixed optical sensor 107 and a metal flag 104 attached to the carousel rotor shaft 60. The flag 104 passes through the optical sensor when the turntable is rotated. This causes the interruption of a light beam which in turn produces an electrical signal readable by the controller 282.

The Home Sensor Mechanism 106 is used to detect when the carousel 20 is at the "home" position. The carousel is turned by the stepper motor 88 until the "home" flag 104 blocks the "home" sensor 106. This sensor only provides approximate positioning, and is used to quickly move to the "home" position.

Between each tube position is a distance equivalent to thirty-two steps of the stepper drive motor 88; i.e. thirty-two motor pulses per each tube step to a next position. Thus, by pulsing the drive motor 88 thirty-two times, the carousel 20 moves one tube position. The Tube Positioning Mechanism 100 consists of an opaque plastic optical sensor disc 96 that has 33 slots placed evenly around its periphery. Each slot represents a tube (or "home") position. This disc is passed through an optical interrupter 103 which produces an electrical signal that directly correlates to the disc slot position within the sensor. The positioning disc 96 has very

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narrow slits 98 that, together with a narrow light beam produced by the optical interrupter 108, yields high rotational positioning precision and accuracy.

The Tube Positioning Sensor 100 is used to first arrive at an accurate "home" position, and then to check positioning accuracy after the stepper motor 88 rotates the carousel 20 to a desired location. In order to avoid tube damage or apparatus jams, the slits 98 are used to check to see that the carousel 20 has moved a tube 22 into a precise tube position, directly centered over the self centering lifter/vortexer mixer 28.

When the MCL apparatus 10 is powered up, assuming its cover is closed, a continuous, rapid stream or burst of pulses is applied to the stepper motor 88, rotating the carousel 20. The CPU controller 282 is signaled by the home position sensor 106 when it becomes blocked by the actuator flag 104 on the actuator member 102 as the carousel 20 is rotated. The signal from the sensor 106 causes the stepper motor 88 to then move more slowly, only one step at a time, until the tube position sensor 100 indicates a slit 98 has passed between its U-shaped arms. Once the leading edge of a slit 98 passes within the sensor 100, the stepper motor 88 is then commanded to look for the next slot edge, while keeping track of the number of pulses it took to get there. The controller 282 then knows the number of pulses necessary to traverse the slit. The total number of pulses are then divided by two (2), the controller 282 reverses the direction of rotation of the stepper motor 88, and steps the number of pulses calculated. This positions the carousel 20 in the center of the slit. All positioning references are then made from this point.

Once the carousel 20 has been positioned accurately at a "home" position, the MCL controller 282 issues pulses to the stepper motor 88 to advance to the next tube position, or a multiple of 32 pulses or steps. Proper positioning is checked with the Tube Positioning Mechanism 100. If a positioning error is not detected, the carousel 20 is rotated in the opposite direction by one half the distance between tubes. This operation is necessary to read the bar code 46 on the carousel 20 placed between tube positions. After a successful read, the direction of rotation is changed again and the carousel 20 is advanced back to its original tube position.

The carousel 20 is "re-homed" if a positioning error is detected. This is to attain the original position accuracy first achieved. The controller 282 then commands the stepper motor 88 to go to a desired tube position.

The tube position and home detect sensors 100 and 106 respectively, provide gross and fine positioning of the carousel 20, with respect to the home position and the position of the next tube 22 to be acted upon. The tube position detect sensor 100 indicates to the CPU controller 282 that the carousel 20 has rotated, from whatever position it was in when stopped, to a slit 98. If the carousel is not in the proper position precisely over the LV mixer 28 to raise a tube and mix its contents, the LV mixer up/down actuator solenoid 310 (FIGS. 2, 10) is not activated. This prevents the LV mixer from moving up, lifting a tube 22 out of the carousel 20 and possibly breaking the tube; thereby splashing outward the contents of the sample, which could be a hazard to the health of the operator. Additionally, this sensor is employed to align the tube to a correct or centered position. This is the reason for the slight adjustment provided for the sensor 100 by means of the previously described adjustment members 108 disposed along the side edge of the plate 74, located adjacent the disc 96.

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This is the "home" reference position and so it must be accurately aligned.

It is understood that "home" position of the carousel is with the projection knob 54 of the carousel support aimed, as in FIG. 3, at the back of the apparatus (to the right in FIG. 2). However, the carousel is loaded onto the carousel support 24 with the projection 54 90° to the left of the home position. With the carousel 20 at the "home" position, the bar code reader/scanner 288 is aligned to read the carousel position bar code as well as the tube identification bar code indicated. This is also the home position of the first tube 22 of the group of tubes on the carousel. Thus, the home position is position zero "0".

The present MCL apparatus 10 is adapted to operate automatically and in an unattended fashion. Control circuitry for each of the multiplicity of operations, as earlier briefly mentioned, is contained on printed circuit board members 278 and 280, (FIGS. 2 and 10). The circuit board 278 supports a CPU controller 282, which controls the mechanical assembly 11 (FIG. 1). The entire content of FIG. 11 is contained in the dotted outline Box 278 of FIG. 10. All of the components above the bus 279, FIG. 11 are contained in the Box 292 of FIG. 10. A 24 VDC power source 293 is used to "power up" the electrical circuitry 278. The circuit board 280 contains the bar code reader electronic control circuitry 284 for a bar code reader 286, including a bar code reader/scanner (not shown). Indicators 290 and buttons 292 are located on the operably associated flow cytometer 19.

A parallel I/O interface 294 is located on the printed circuit board 278 and its electrical input signals come from the flow cytometer 19. This interface provides access from the flow cytometer to the CPU controller 282. A scanner relay 296 and a scanner power input 298 operate with the interlock detect 300 to automatically turn "off" the bar code reader/scanner (not shown), to prevent laser beam emission, which if unchecked could cause personnel injury.

The interlock detect 300 also indicates the status of the sample tube support head 32 and sample aspirating member 34, which must be in operative position for the apparatus 10 to operate. The scanner power input 298 energizes the bar code scanner/reader (not shown) from a serial interface connector 302 on 278. The cover interlock detect 304 includes switches which are wired in series, such that if one switch fails the other will trip and shut off the power.

The probe detect chip 262, the head detect chip 250, the LV up/down detect chip 240 and the carousel in/out detect chip 294 all refer to previously described mechanical apparatus illustrated in FIG. 9. These detectors indicate the status of each moveable part of the apparatus 10. For example, if the sample probe 34 should become stuck in a half way up position, this is considered a fault, and appropriate technician action is required.

The sample head detect sensor 226 primarily detects that a tube 22 has been loaded. Its secondary function is operative during any scanning sequence or tube loading routine, when the sample probe 214 is aspirating, such that if the tube 22 should break or should be flung out of the carousel 20, this sensor 226 will instantly shut down the total operation. The flow cytometer then will "ask" for a status report and the MCL 10 will "report" the detect sensor head fault.

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The LV up/down detect sensor 224 is the tube position monitor. This detector indicates that the tube sensor actuator or flag 242 reached a certain raised position at a predetermined time monitored by the software program. The software assembly 17 keeps track of the time and the status of this sensor 224.

The carousel in/out detect sensor 230 (FIG. 5) detects the horizontal position (arrow 27) of the carousel 20 with respect to the self centering lifter vortexer/mixer 28. If the carousel 20 is not in the proper position for the vortexer mixer 28 to raise a tube 22 from the carousel 20 and mix the tube contents, the vortexer/mixer is not activated.

Four solenoid operated valves, identified as: carousel in/out actuator solenoid 306, sample probe actuator solenoid 308, LV up/down mixer solenoid 310, and sample tube rotator solenoid 312 are shown at the lower left in FIG. 2. The stepper motor 88 is controlled through a power driver 314 and motor control circuitry 316. The LV motor 128, which actuates the lifter vortexer/mixer 28, is controlled by the motor driver circuitry 318. A serial interface 320 provides the interconnection for an unused auxiliary serial port.

Referring now to FIG. 11, there is shown a reset block or chip 322, which detects when the supply voltage drops below a fixed, pre-programmed level. If the voltage does thus drop, the reset chip 322 resets the status of the whole circuit board 278. This action avoids any erroneous conditions or uncertain logic levels which might be caused by low supply voltage (from source 293 FIG. 10) solely during "power up". If the block 322 is not reset, this indicates to an MPU processor 324 that the electronic circuitry is at the proper voltage level so that the MPU processor 324 can execute commands accurately and in an ordered sequence. A clock chip 326 provides a clock frequency for microprocessor support, in well known fashion. An address decoder 328, which can be a PAL, is configured to have a linear addressing function. Address and data latches 330 provide additional addressing access to memory. An E prom 332 and two 32K RAM chips 334 and 336 provide the current capability for the electrical assembly 13.

In FIG. 11, the multiple output lines from the address decoder 328 provide means to address many locations in the processor board system. The electrical assembly 13 must be given a specific command instruction to access a specific address therein. The two RAM chips 334 and 336 introduce various operational states into and out of the MCL apparatus 10 during operation. A timer 338 is operationally based on the cycle of the clock 326, to provide an accurate time base to run certain time sequences used in the electrical control assembly 13.

Configuration switches 340 are a plurality of LED/dip switches used to set the MCL apparatus 10 into its various modes of operation. For example, by appropriate configuration switch setting, the MCL apparatus 10 can take its commands solely from the flow cytometer 19; or it can be operated from a lap top computer (not shown) for test purposes or diagnostic routines. Command inputs solely from the flow cytometer 19 are through a parallel control interface line 342. Other type of command inputs can be introduced via a serial control interface line 344.

An interrupt controller 346 monitors other event/signals entering inputs from other ports of the circuit board 278. These signals can come from inside or outside the MCL apparatus 10, or from other switches. The

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main purpose of this chip 346 is to locate the interrupt, whatever or wherever it is, and to inform the electrical assembly, so that appropriate rectifying action can be taken in an orderly fashion. Two serial I/O's 348 and 350 are located in interfaces 302 and 320 and perform dedicated functions. The serial I/O 348 is completely dedicated to the bar code reader scanner 286; all communications to the scanner go through this port 348. The second I/O 350 is connected to the serial control interface line 344, for future use.

A first parallel I/O 352 is connected and configured to provide inputs to the parallel control interface line 342. This I/O interfaces with any of the many switches used throughout the electrical assembly 13. This I/O accepts all of the input data and is dedicated to the flow cytometer 19. The second parallel I/O 354 interfaces with buffer and driver circuits 356. The flow cytometer 19 has all of its communications with the MCL apparatus 10 through the I/O 354.

Description of an Operational Cycle of the MCL Apparatus 10

As is typical in the use of flow cytometry, the human operator pre-programs the cytometer 19 via the work station 15 to set up a protocol for conducting one or more tests upon a sample, for example from a tube 22. Since the present invention and its example of the carousel 20 can hold up to thirty-two sample tubes, the human operator can pre-program the work protocol or program to be done on any and/or all of the samples on the carousel soon to be loaded into the MCL apparatus 10. Essential to the operator input protocol is designation of the bar code indicia label 42 of the sample tube upon which certain specific tests are to be conducted and its pocket position 46 in the carousel. Also, if more than one carousel is to be employed, its designator is to be inputted into the protocol. The protocol can be input prior to or after the carousel is mounted onto the stub shaft 60 on the plate 74. Then the MCL 10 apparatus cover is closed, so that the MCL can enable total automatic system operation.

With the flow cytometer 19 "ON", the MCL apparatus 10 automatically is energized. There are a number of initialization procedures and processes that take place automatically. The apparatus 10 resets the CPU controller 282 to ensure that it is in the proper state to begin operation. The apparatus 10 now is in an idling condition waiting for commands. The flow cytometer 19 has checked all its sensors, all processors, etc. and has initiated a software reset. The MPU processor 324 of the MCL 10 initiates a reset of the scanner board 286 and simultaneously starts putting all the mechanisms of the mechanical assembly 11 in their proper positions. The carousel support 24 is moved outward (arrow 27) into the "load" position, which is the standard position for loading and unloading the carousel 20 carrying its tubes 22. The apparatus 10 now waits for the next command from the flow cytometer 19. At this point, the flow cytometer 19 can send a tube load command and designate which tube, by number, is to be loaded.

Assuming at this point that the carousel is loaded into the apparatus and the cover of the apparatus 10 is closed, the operator presses an "auto" start button on the flow cytometer 19. The cytometer 19 now sends the tube load command to the MCL apparatus 10. The carousel 20 turns around counter clockwise. First, the carousel position 44 is identified, then the carousel number is read for identifying the proper carousel 20, and

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then the number 42 tube 22 to be tested is identified. The carousel now is moved forwardly or “in” to the proper tube position and the tube is centered. The finger 30 extends outwardly and oscillates back and forth against the side of the tube, rotating the tube circularly. During this rotating portion of the cycle, the bar code reader 286 reads the bar code label 42 on the tube, identifying the specific tube for a specific test. The bar code reader electronic control circuitry 284 is preprogrammed to provide a number of reads. When it has produced three so-called “good reads”, the reader circuitry 284 indicates a valid read. This identifies the information/data on the tube. This data is then fed back to the flow cytometer 19, where a system check of the data then is made. The tube 22 is still in pocket 40 in the carousel 20 at this point. This ends the tube load command.

The flow cytometer 19 now issues a “tube raise command”. The finger 30 comes out again and this time the finger 30 is utilized as a support device for the tube 22. As the tube 22 is lifted from the carousel 20 (pushed upward from the pocket 40) by the lifter vortexer 28, the finger 30 prevents the tube from tilting or wobbling out of the vertical orientation. The lift command is sent to the LV up/down actuator solenoid 310, which then raises the tube 22 to engage the open top of the tube with the sample tube support head 32. The distance the tube travels during the “up” excursion depends upon the length of the tube. The LV up/down vortexer 28 now is actuated to rotate the tube. The motor driver 318 switch is turned “on” and the motor 128 causes the LV mixer 28 to rotate the tube 22 and resuspend or mix its contents. When the mixing is completed, the probe actuator solenoid 308 is energized, bringing the elongated probe 214 down into the tube. The probe detect 262 is operable to indicate the position of the probe 214 with respect to the sample head 32. The tip of the probe 214 enters the tube 22 and goes almost to the bottom of the tube. The tube now is pressurized by compressed air, which enters the tube via the orifice or passageway 192 in the sample head 32. The air forces a specific volume of fluid sample vertically upwardly through the probe 214 into the flow cytometer 19 during a predetermined time sequence. Then, applied vacuum to the orifice or passageway 194 sucks up any refuse or liquid drops that remain on the probe 214. The sample tube support head 32 and the probe tip are washed by fluid which is introduced via the pressure passageway 192 and simultaneously the wash and waste fluids are sucked out via the vacuum passageway 194, thus cleaning that portion of the apparatus. Next, the probe 214 is lifted vertically upward to its fully retracted position. The tube 22 is lowered by the LV vortexer mixer 28 and the sample head 32, which is spring biased upwardly by the spring 212, is forced up into a retracted home position. If the tube 22 happens to stick within the sample head 32, there is a “shake” command through the motor control 316, whereby the tube 22 is now shaken or vibrated prior to being returned to the carousel pocket from which it came. At this point, the apparatus 10 waits for another command from the flow cytometer 19. The cycle of operation is completed and the carousel 20 moves outwardly and rotates to the load position. The MCL apparatus 10 now waits for the next operational command.

We claim:

1. Apparatus for automatically and selectively transferring multiple, randomly associated hematological

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samples from a group of samples to an operably associated cytometric apparatus comprising,

at least one sample container, each containing a preselected, individually identifiable hematological sample,

sample container carrier means for moving said sample containers on a path of movement including means for supporting a plurality of sample containers thereon,

movable sample receiving means for pressurizing said sample prior to passing said sample to a cytometric apparatus,

means for moving said sample container carrier means normal to the path of movement of said sample receiving means,

sample resuspending and mixing means operably associated with said sample container carrier means for automatically removing a sample container from said carrier means and engaging said sample container with said sample receiving means, so that activation of said sample resuspending and mixing means resuspends and mixes said sample, a movable member having means for contacting said sample container and vertically lifting said container from said carrier means into temporary sealing engagement of said sample container with said sample receiving means and for translating the vertical lifting movement into circular, vortexing movement so as to vortex the contents of said sample container, said movable member including a vertically disposed member having a slidable counter balance affixed at one end, permitting said movable member to automatically adjust its position from off-center to center as it is moved with respect to a sample container,

said sample container carrier means including an opening in said supporting means below each sample container and said movable member contacting means moves through said opening to lift said container from said carrier means, and

aspirating means operably engageable with said sample receiving means for delivering said sample from said sample container to said cytometric apparatus in response to the pressurizing of said sample.

2. The apparatus in accordance with claim 1 wherein said sample container carrier includes preselected identification indicia to identify each individual sample container.

3. The apparatus in accordance with claim 1 wherein said sample container carrier means includes a demountable, rotatable carousel including drive engaging means for engaging carousel and said container carrier means.

4. The apparatus in accordance with claim 3, further including drive means for coupling to said drive engaging means and orienting means for orienting said carousel in load and home positions.

5. The apparatus in accordance with claim 4 wherein said orienting means includes position and indicating means for rotating said sample container carrier means to move a selected sample container into engagement with said sample resuspending means and for moving said selected sample container into engagement with said sample receiving means.

6. The apparatus in accordance with claim 1 wherein said sample receiving means includes integral back flushing means for removing residual sample therefrom.

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7. The apparatus in accordance with claim 1 further including means horizontally movable into and out of engagement with a sample container for automatically rotating said sample container into a position for identifying individual indicia thereon and for supporting said sample container when said sample container is removed from said carrier means for engagement with said sample receiving means.

8. The apparatus in accordance with claim 7 wherein said means for rotating and supporting said sample container comprises an elongated, pivoted link carrying a resilient sample container engaging member at a free end thereof.

9. The apparatus in accordance with claim 1 further including drive means for moving said movable member vertically up and down.

10. The apparatus in accordance with claim 1 including signal generating means for indicating the position of said container carrier means with respect to said movable member so as to coaxially position said movable member beneath a sample container, enabling said sample container to be raised vertically clear of said carrier means to bring said sample container into sealing engagement with said sample receiving means.

11. The apparatus in accordance with claim 1 wherein an individual electro-optical position sensor is operably associated with each one of said container carrier means, said sample receiving means, said sample resuspending and mixing means and said aspirating means; each of said position sensors including an elongated opaque member operably positioned to interrupt and activate a respective sensing member as said carrier means, said sample receiving means, said sample resuspending and mixing means and said aspirating means is moved during operation of said apparatus.

12. An apparatus for selectively transferring samples from a group of samples, comprising:

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a plurality of sample containers, each container including a base end and a sample loading and aspiration end;

a sample carrier including means for holding at least said base end of said plurality of sample containers and means for moving said sample containers to a mixing station;

means for supporting said sample loading and aspiration end of said sample containers in said aspiration location spaced from said sample carrier;

sample resuspending and mixing means operably associated with said sample container carrier for removing a sample container from said carrier means and engaging said sample container with said supporting means, so that activation of said sample resuspending and mixing means resuspends and mixes said sample, a movable member having means for contacting said sample container and vertically lifting said container from said carrier into temporary sealing engagement of said sample container with said supporting means and for translating the vertical lifting movement into circular, vortexing movement so as to vortex the contents of said sample container, said movable member including a vertically disposed member having a slidable counter balance affixed at one end, permitting said movable member to automatically adjust its position from off-center to center as it is moved with respect to a sample container, and

said sample container carrier including an opening in said base end holding means below each sample container and said movable member contacting means moves through said opening to lift said container from said carrier.

13. The apparatus in accordance with claim 12 including aspirating means operably engagable with said supporting means for delivering said sample from said sample container.

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EXHIBIT

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

(Case No. 97,008-X)

In re Application of:)	
)	
COPELAND, et. al)	
)	Group Art Unit: Not yet assigned
Serial No.: Not yet assigned)	
)	Examiner: Not yet assigned
Filed: January 22, 2002)	
)	
For: Automated Biological)	
Reaction Apparatus)	

Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Dear Sir:

Please enter this preliminary amendment for the above-referenced case.

IN THE SPECIFICATION

Please amend lines 3-5 at page 1 to read as follows:

This is a continuation of application Serial No. 09/452,309, filed on December 1, 1999, pending, which is a continuation of application Serial No. 08/906,678, filed August 5, 1997, abandoned, which is a continuation of application Serial No. 08/479,415, filed June 6, 1995, U.S. Patent No. 5,654,200, which is a division of application Serial No. 352,966, filed December 9, 1994, U.S. Patent No. 5,595,707, which is a continuation of application Serial No. 924,052, filed August 31, 1992, abandoned, which is a continuation-in-part of application Serial No. 488,601, filed March 2, 1990, abandoned.

After page 12, line 10, please insert the following paragraph:

FIG. 34 is a schematic of a jet drain for draining liquid from an upper surface of a slide.

At page 41, line 3-10, please amend the paragraph to read:

Immunohistological methods for which the apparatus of this invention are particularly suitable are described in concurrently filed, commonly assigned patent application Serial No. 07/488,601, filed March 2, 1990, now abandoned (Attorney Docket No. 193.0007), the entire contents of which are hereby incorporated by reference. A typical immunohistological method, as carried out with the apparatus of this invention includes the following steps.

IN THE CLAIMS:

Please cancel claim 1 without prejudice. Please add the following claims 72-98 as follows. A marked up version of the amended claims, to show all the changes, is attached hereto on pages separate from the amendment in accordance with 37 CFR 1.121(c)(1)(ii).

72. A method of dispensing reagents onto a slide, the method comprising the steps of:
providing at least one reagent container;
providing at least one slide on a slide support;
automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container;
automatically determining whether reagent in the reagent container should be dispensed onto the slide; and

dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide.

73. The method of claim 72, wherein the step of automatically determining whether reagent in the reagent container should be dispensed onto the slide includes identifying information from the slide.

74. The method of claim 73, further comprising the steps of:
determining a position of the slide; and
correlating the position of the slide with the information identifying the slide.

75. The method of claim 73, wherein the information identified from the slide includes a slide barcode.

76. The method of claim 75, wherein the step of identifying information from the slide includes reading the slide barcode.

77. The method of claim 76, further comprising the steps of:
determining a position of the slide; and
correlating the position of the slide with the slide barcode.

78. The method of claim 77, wherein the step of determining a position of the slide includes determining a position of the slide relative to a home position.

79. The method of claim 72, wherein the information associated with the reagent container includes a reagent barcode.

80. The method of claim 79, wherein the reagent barcode is placed on the reagent container.

81. The method of claim 72, wherein the step of automatically identifying the reagent container using a computer includes the steps of:

- providing a bar code reader;
- reading a reagent bar code placed on the reagent container using the bar code reader thereby
- acquiring reagent information; and
- sending the reagent information to the computer.

82. The method of claim 81, further comprising the steps of:

- determining position information for the reagent container; and
- sending the position information to the computer.

83. The method of claim 82, wherein a reagent carousel supports the reagent container and wherein the step of determining position information for the reagent container includes homing the reagent carousel and determining an indexed position of a motor drive for the reagent container.

84. The method of claim 81, wherein the reagent bar code identifies the reagent in the reagent container.

85. The method of claim 84, wherein the step of automatically identifying the reagent container using a computer is performed at a beginning of a slide treatment operation.

86. The method of claim 85, wherein the step of automatically identifying the reagent container using a computer further includes correlating a position of the reagent container with the reagent carousel.

87. The method of claim 81, wherein the reagent container is in a reagent carousel and wherein the step of automatically identifying reagent further includes the step of rotating the reagent carousel so that the reagent bar code on the reagent container is read by the bar code reader.

88. The method of claim 72, further comprising the step of moving the reagent container and the slide support relative to one another to position the reagent container over the slide.

89. The method of claim 88, wherein a reagent carousel supports the reagent container and wherein the step of moving the reagent container and the slide support relative to one another includes moving a drive plate which supports the reagent carousel to place the reagent container in a reagent delivery zone.

90. The method of claim 72, wherein the step of dispensing the reagent in the reagent container onto the slide includes the step of pressuring the reagent container thereby metering a volume of reagent onto the slide.

91. The method of claim 90, wherein the step of pressuring the reagent container includes activating an air cylinder to move downward into positive contact with the reagent container.

92. A method of dispensing reagents onto a slide, the method comprising the steps of:
providing at least one reagent container;
providing at least one slide on a slide support;
automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container;
automatically determining whether the reagent in the reagent container should be dispensed onto the slide;
moving the reagent container and the slide support relative to one another to position the reagent container over the slide; and
dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide.

93. The method of claim 92, wherein the information associated with the reagent container includes a reagent barcode.

94. The method of claim 93, wherein the reagent barcode is placed on the reagent container.

95. The method of claim 92, wherein the step of automatically identifying the reagent container using a computer includes the steps of:

providing a bar code reader;

reading a reagent bar code placed on the reagent container using the bar code reader; and

sending information from the reading of the reagent bar code to the computer.

96. The method of claim 95, wherein the reagent container is supported on a reagent carousel and wherein the step of moving the reagent container and the slide support relative to one another includes moving a drive plate which supports the reagent carousel to place the reagent container in a reagent delivery zone.

97. The method of claim 92, wherein the step of dispensing the reagent in the reagent container onto the slide includes the steps of:

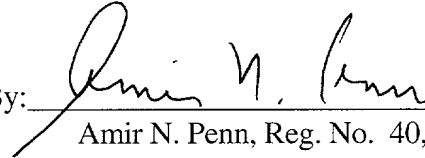
pushing downward on the reagent container; and

applying a metered volume of reagent onto the slide.

98. The method of claim 97, wherein the step of pushing downward on the reagent container includes activating an air cylinder to move downward in order to push the reagent container.

Respectfully submitted,

McDonnell Boehnen Hulbert & Berghoff

By: 
Amir N. Penn, Reg. No. 40,767
Attorney for Applicant

DATED: 1/22/02

APPENDIX UNDER 37 CFR 1.121(c)

IN THE SPECIFICATION

Please amend lines 3-5 at page 1 to read as follows:

This is a continuation of application Serial No. 09/452,309, filed on December 1, 1999, pending, which is a continuation of application Serial No. 08/906,678, filed August 5, 1997, abandoned, which is a continuation of application Serial No. 08/479,415, filed June 6, 1995, U.S. Patent No. 5,654,200, which is a division of application Serial No. 352,966, filed December 9, 1994, U.S. Patent No. 5,595,707, which is a continuation of application Serial No. 924,052, filed August 31, 1992, abandoned, which is a continuation-in-part of application Serial No. 07/488,601, filed March 2, 1990, abandoned.

After page 12, line 10, please insert the following paragraph:

FIG. 34 is a schematic of a jet drain for draining liquid from an upper surface of a slide.

At page 41, line 3-10, please amend the paragraph to read:

Immunohistological methods for which the apparatus of this invention are particularly suitable are described in concurrently filed, commonly assigned patent application Serial No. [_____] 07/488,601, filed March 2, 1990, now abandoned (Attorney Docket No. 193.0007), the entire contents of which are hereby incorporated by reference. A typical immunohistological method, as carried out with the apparatus of this invention includes the following steps.

IN THE CLAIMS

72. (New claim) A method of dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container;

providing at least one slide on a slide support;

automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container;

automatically determining whether reagent in the reagent container should be dispensed onto the slide; and

dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide.

73. (New claim) The method of claim 72, wherein the step of automatically determining whether reagent in the reagent container should be dispensed onto the slide includes identifying information from the slide.

74. (New claim) The method of claim 73, further comprising the steps of:
determining a position of the slide; and
correlating the position of the slide with the information identifying the slide.

75. (New claim) The method of claim 73, wherein the information identified from the slide includes a slide barcode.

76. (New claim) The method of claim 75, wherein the step of identifying information from the slide includes reading the slide barcode.

77. (New claim) The method of claim 76, further comprising the steps of:
determining a position of the slide; and
correlating the position of the slide with the slide barcode.

78. (New claim) The method of claim 77, wherein the step of determining a position of the slide includes determining a position of the slide relative to a home position.

79. (New claim) The method of claim 72, wherein the information associated with the reagent container includes a reagent barcode.

80. (New claim) The method of claim 79, wherein the reagent barcode is placed on the reagent container.

81. (New claim) The method of claim 72, wherein the step of automatically identifying the reagent container using a computer includes the steps of:

providing a bar code reader;

reading a reagent bar code placed on the reagent container using the bar code reader thereby
acquiring reagent information; and

sending the reagent information to the computer.

82. (New claim) The method of claim 81, further comprising the steps of:
determining position information for the reagent container; and
sending the position information to the computer.

83. (New claim) The method of claim 82, wherein a reagent carousel supports the reagent container and wherein the step of determining position information for the reagent container includes homing the reagent carousel and determining an indexed position of a motor drive for the reagent container.

84. (New claim) The method of claim 81, wherein the reagent bar code identifies the reagent in the reagent container.

85. (New claim) The method of claim 84, wherein the step of automatically identifying the reagent container using a computer is performed at a beginning of a slide treatment operation.

86. (New claim) The method of claim 85, wherein the step of automatically identifying the reagent container using a computer further includes correlating a position of the reagent container with the reagent carousel.

87. (New claim) The method of claim 81, wherein the reagent container is in a reagent carousel and wherein the step of automatically identifying reagent further includes the step of rotating the reagent carousel so that the reagent bar code on the reagent container is read by the bar code reader.

88. (New claim) The method of claim 72, further comprising the step of moving the reagent container and the slide support relative to one another to position the reagent container over the slide.

89. (New claim) The method of claim 88, wherein a reagent carousel supports the reagent container and wherein the step of moving the reagent container and the slide support relative to one another includes moving a drive plate which supports the reagent carousel to place the reagent container in a reagent delivery zone.

90. (New claim) The method of claim 72, wherein the step of dispensing the reagent in the reagent container onto the slide includes the step of pressuring the reagent container thereby metering a volume of reagent onto the slide.

91. (New claim) The method of claim 90, wherein the step of pressuring the reagent container includes activating an air cylinder to move downward into positive contact with the reagent container.

92. (New claim) A method of dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container;

providing at least one slide on a slide support;

automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container;

automatically determining whether the reagent in the reagent container should be dispensed onto the slide;

moving the reagent container and the slide support relative to one another to position the reagent container over the slide; and

dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide.

93. (New claim) The method of claim 92, wherein the information associated with the reagent container includes a reagent barcode.

94. (New claim) The method of claim 93, wherein the reagent barcode is placed on the reagent container.

95. (New claim) The method of claim 92, wherein the step of automatically identifying the reagent container using a computer includes the steps of:

providing a bar code reader;

reading a reagent bar code placed on the reagent container using the bar code reader; and

sending information from the reading of the reagent bar code to the computer.

96. (New claim) The method of claim 95, wherein the reagent container is supported on a reagent carousel and wherein the step of moving the reagent container and the slide support relative to one another includes moving a drive plate which supports the reagent carousel to place the reagent container in a reagent delivery zone.

97. (New claim) The method of claim 92, wherein the step of dispensing the reagent in the reagent container onto the slide includes the steps of:

pushing downward on the reagent container; and
applying a metered volume of reagent onto the slide.

98. (New claim) The method of claim 97, wherein the step of pushing downward on the reagent container includes activating an air cylinder to move downward in order to push the reagent container.

EXHIBIT

12



APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/054,535	01/22/2002	Keith G. Copeland	97,008-X	7198

20306 7590 10/30/2002

MCDONNELL BOEHNNEN HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
SUITE 3200
CHICAGO, IL 60606

EXAMINER

BEX, PATRICIA K

ART UNIT	PAPER NUMBER
----------	--------------

1743

DATE MAILED: 10/30/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

10/054,535

Applicant(s)

COPELAND ET AL.

Examiner

P. Kathryn Bex

Art Unit

1743

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 18 October 2002.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 72-98 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 72-98 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 22 January 2002 is/are: a) ☐ accepted or b) ☒ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- 11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.
- If approved, corrected drawings are required in reply to this Office action.
- 12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

- 13) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.
- 14) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).
- a) ☐ The translation of the foreign language provisional application has been received.
- 15) ☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

- 1) ☐ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 1,4.
- 4) ☐ Interview Summary (PTO-413) Paper No(s). _____.
- 5) ☐ Notice of Informal Patent Application (PTO-152)
- 6) ☐ Other: _____.

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DETAILED ACTION

1. The cancellation of claims 1-71 is acknowledged and has been entered into the record.

Drawings

2. The drawings are objected to because Figures 18A-18C are not referenced in the Brief Description of Drawing section. A proposed drawing correction or corrected drawings are required in reply to the Office action to avoid abandonment of the application. The objection to the drawings will not be held in abeyance.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

4. The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
 2. Ascertaining the differences between the prior art and the claims at issue.
 3. Resolving the level of ordinary skill in the pertinent art.
 4. Considering objective evidence present in the application indicating obviousness or nonobviousness.
5. Claims 72-87, 90-91 are rejected under 35 U.S.C. 103(a) as being unpatentable over Heidt *et al* (USP 5,250,262) in view of Sakurada (USP 4,346,056).

Heidt *et al* teach a chemical analyzer utilizing a method of dispensing reagents (e.g.

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serum) onto a slide 71. The method comprises the steps of providing a reagent container containing serum and providing a slide on a slide carousel 50. Additionally, Heidt *et al* teach automatically determining whether the serum in the pipetter 16 should be dispensed onto the slide by reading, via BCR 158, the information contained on the barcode 86 of the slide (column 15, lines 20-24, column 30, lines 33-49). Additionally, Heidt *et al* teaches determining the position of a slide relative to a home position (column 26, lines 40-68.) Heidt *et al* fail to teach the step of automatically identifying the reagent using a computer, based on information associated with the reagent container.

Sakurada does teach the step of automatically identifying the reagent using a computer, based on information associated with the reagent container 7. Sakurada discloses providing a bar-code reader 31 for reading a reagent bar-code 33 on the reagent container and sending the reagent information to a control device 24 (column 3, line 12- column 4, line 38). Additionally, Sakurada teaches determining the position of the reagent container on a carousel and sending that position information to the computer (column 5, line 67- column 6, line 5, Figs. 3, 5-8).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time of the claimed invention to have substituted the manual method of Heidt *et al* of inputting reagent information into the computer with the step of automatically inputting the reagent information into a computer in order to reduce the possibility of data entry errors associated with manual data entry. Moreover, it has been held that broadly providing a mechanical or automatic means to replace manual activity which has accomplished the same result involves only routine skill in the art. *In re Venner*, 120 USPQ 192.

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6. Claims 88-89, 92-98 are rejected under 35 U.S.C. 103(a) as being unpatentable over Heidt *et al* (USP 5,250,262) and Sakurada (USP 4,346,056), as applied to claim 72, in further view of Rokugawa (USP 4,844,868).

Heidt *et al* and Sakurada as previously discussed above do not teach the step of moving the reagent container and slide support relative to one another to position the reagent container over the slide. Rokugawa does teach an apparatus for delivering reagent to reaction containers wherein a plurality of reagents 68 are supported on a reagent carousel 64 which is positioned above a reaction carousel. A reagent delivery actuator means 100 for engaging a reagent container and initiating delivery of reagent (Fig. 1). The positioning of the reagent delivery system over the reaction carousel reduces distribution time and reduces the possibility that cross contamination will occur (column 6, lines 8-14).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time of the claimed invention to have positioned the reagent delivery system of Heidt *et al* and Sakurada over the reaction carousel, as taught by Rokugawa, in order to reduce distribution time and possibility that cross contamination will occur.

Conclusion

7. No claims allowed.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to P. Kathryn Bex whose telephone number is (703) 306-5697. The examiner can normally be reached on Mondays-Thursdays, alternate Fridays from 6:00 am to 3:30 pm EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jill Warden can be reached on 308-4037.

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Page 5

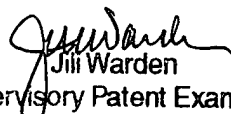
Art Unit: 1743

The fax number for the organization where this application or proceeding is assigned is (703) 872-9310 for official papers prior to mailing of a Final Office Action. For after-Final Office Actions use (703) 872-9311. For unofficial or draft papers use fax number (703) 305-7719. Please label all faxes as official or unofficial. The above fax numbers will allow the paper to be forwarded to the examiner in a timely manner.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0661.



P. Kathryn Bex
Patent Examiner
AU 1743
October 29, 2002


Jili Warden
Supervisory Patent Examiner
Technology Center 1700

FORM PTO-1449
(Rev. 2-32)U.S. Department of Commerce
Patent and Trademark Office

Atty. Docket No.

Serial No.

97,008-X

Unassigned

RECEIVED

MAR 21 2002

Applicant:

GROUP 3600

Copeland et al.

Filing Date:

Group:

January 22, 2002

1745
UnassignedINFORMATION DISCLOSURE
STATEMENT BY APPLICANT

(Use several sheets if necessary)

U.S. PATENT DOCUMENTS

Examiner Initial		Document Number	Date	Name	Class	Subclass	Filing Date if Appropriate
OB	AA	4,298,571	11/3/81	DiFulvio et al.			
	AB	4,346,056	08/24/82	Sakurada			
	AC	4,844,868	07/04/89	Rokugawa			
	AD	4,919,887	4/24/90	Wakatake			
	AE	4,935,875	06/19/90	Shah et al.			
	AF	4,961,906	10/09/90	Andersen et al.			
	AG	4,985,206	01/15/91	Bowman et al.			
	AH	5,075,079	12/24/91	Kerr et al.			
IB	AI	5,180,606	01/19/93	Stokes et al.			

FOREIGN PATENT DOCUMENTS

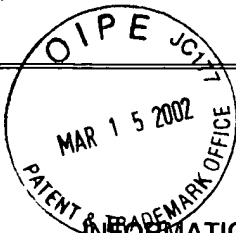
		Document Number	Date	Country	Class	Subclass	Translation Yes No
CH	BA	87/00086	1/15/87	PCT			
BY	BC	2 216 259	3/30/88	GB			

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc).

W	CA	Stark et al., An Automated Device of Immunocytochemistry, Journal of Immunological Methods, 1988, Elsevier, 107, pp.89-92.					
EXAMINER					DATE CONSIDERED		
Kath Buss					10/28/97		

EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication.

FORM PTO-1449
(Rev. 2-32)



U.S. Department of Commerce
Patent and Trademark Office

Atty. Docket No.

97,008-X

Serial No.

Unassigned

INFORMATION DISCLOSURE
STATEMENT BY APPLICANT
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Applicant:

Copeland et al.

MAR 21 2002

GROUP 3600

Filing Date:

January 22, 2002

Group:

1743
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U.S. PATENT DOCUMENTS

Examiner Initial		Document Number	Date	Name	Class	Subclass	Filing Date if Appropriate
CS	AJ	5,232,664	08/03/93	Krawzak et al.			
	AK	5,356,595	10/18/94	Kanamori et al.			
	AL	5,424,036	06/13/95	Ushikubo			
	AM	5,425,918	06/20/95	Healey et al.			
	AN	5,439,645	08/08/95	Saralegui et al.			
	AO	5,439,649	08/08/95	Tseung et al.			
	AP	5,645,114	07/08/97	Bogen et al.			
	AQ	5,654,200	08/05/97	Copeland et al.			
	AR	5,947,167	09/07/99	Bogen et al.			
CS	AS	6,193,933	02/27/01	Sasaki et al.			

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Subclass	Translation Yes No

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc).

EXAMINER	DATE CONSIDERED
<i>Koch</i>	<i>10/28/02</i>

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PTO/SB/08A (08-00)

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U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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Substitute for form 1449A/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)				Complete if Known	
				Application No.	10/054,535
				Filing Date:	January 22, 2002
				First Named Inventor	Copeland
				Group Art Unit	3612 1743
				Examiner Name	unassigned Ben
Sheet 1 of 5	Attorney Docket No. 97,008-X				

U.S. PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines Where Relevant Passages or Figures Appear
		Number	Kind Code ² (if known)			
Q		3,219,416	A	Natelson	11-23-1965	
		3,398,935	A	Livesey, <i>et al.</i>	8-27-1968	RECEIVED
		3,574,064	A	Binnings, <i>et al.</i>	4-6-1971	OCT 21 2002
		3,772,154	A	Isenberg <i>et al.</i>	11-13-1973	GROUP 3800
		3,853,092	A	Amos <i>et al.</i>	12-10-1974	
		3,854,703	A	Gibbs <i>et al.</i>	12-17-1974	
		4,013,038	A	Rogers <i>et al.</i>	03-22-1977	RECEIVED
		4,092,952	A	Wilkie <i>et al.</i>	06-06-1978	OCT 23 2002
		4,113,437	A	Duff <i>et al.</i>	9-12-1978	TC 1700
Q		4,200,056	A	Johnson	4-29-1980	

FOREIGN PATENT DOCUMENTS								
Examiner Initials*	Cite No. ¹	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines Where Relevant Passages or Figures Appear	T ⁶
		Office ³	Number ⁴	Kind Code ⁵ (if known)				
Q		FR	2239167	A6	Ministere de L'Agriculture, Service Veterinaire	7-26-1973		
		FR	2258122	A1	Etablissements Valois SA	12-9-1983		
		WO	8503571	A1	William C. Hulette	8-15-1985		
		WO	8802865	A1	Terence Weston	04-21-1988		
Q								

Examiner Signature	<i>John Ben</i>	Date Considered	10/2/02
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¹ Unique citation designation number. ² See attached Kinds of U.S. Patent Documents. ³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). ⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁶ Applicant is to place a check mark here if English translation is attached.

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet

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of

5

Complete if Known

Application No.	10/054,535
Filing Date:	January 22, 2002
First Named Inventor	Copeland
Group Art Unit	3612-1743
Examiner Name	unassigned <i>Key</i>
Attorney Docket No.	97,008-X

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No. 1	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines Where Relevant Passages or Figures Appear
		Number	Kind Code ² (if known)			
<i>CB</i>		4,200,607	A	Suzuki	4-29-1980	
		4,245,967	A	Busselet	1-20-1981	
		RE 30,730	A	Duff	9-1-1981	RECEIVED
		4,298,571	A	DiFulvio <i>et al.</i>	11-3-1981	OCT 21 2002
		4,406,547	A	Aihara	9-27-1983	
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		4,455,280	A	Shinohara <i>et al.</i>	6-19-1984	
		4,528,159	A	Liston	7-9-1985	
		4,567,748	A	Klass <i>et al.</i>	2-4-1986	RECEIVED OCT 23 2002 TC 1700
		4,585,622	A	Bowe <i>et al.</i>	4-29-1986	
		4,656,006	A	Assmann <i>et al.</i>	4-7-1987	
		4,664,526	A	Scheffler <i>et al.</i>	5-12-1987	
		4,681,741	A	Hanaway	7-21-1987	
<i>CB</i>		4,708,886	A	Nelson	11-24-1987	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No. 1	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines Where Relevant Passages or Figures Appear	T ⁶
		Office ³	Number ⁴	Kind Code ⁵ (if known)				
Examiner Signature	<i>Kathryn</i>					Date Considered	<i>10/25/02</i>	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ Unique citation designation number. ² See attached Kinds of U.S. Patent Documents. ³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). ⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁶ Applicant is to place a check mark here if English translation is attached.

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet

3

of

5

Complete if Known

Application No.

10/054,535

Filing Date:

January 22, 2002

First Named Inventor

Copeland

Group Art Unit

3612 1743

Examiner Name

unassigned (Ary)

Attorney Docket No.

97,008-X

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No. 1	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines Where Relevant Passages or Figures Appear
		Number	Kind Code ² (if known)			
C3		4,764,342	A	Kellin <i>et al.</i>	8-16-1988	
		4,774,055	A	Wakatake <i>et al.</i>	9-27-1988	
		4,781,891	A	Galle <i>et al.</i>	11-1-1988	
		4,795,710	A	Muszak <i>et al.</i>	1-3-1989	
		4,815,978	A	Mazza <i>et al.</i>	3-28-1989	
		4,849,177	A	Jordan	7-18-1989	
		4,865,811	A	Newton <i>et al.</i>	9-12-1989	
		4,900,513	A	Barker <i>et al.</i>	2-13-1990	
		4,919,887	A	Wakatake	4-24-1990	
		4,933,147	A	Hollar <i>et al.</i>	6-12-1990	
		4,943,415	A	Przybylowicz <i>et al.</i>	7-24-1990	
		4,965,049	A	Lillig <i>et al.</i>	10-23-1990	
C5		4,988,482	A	Weston	1-29-1991	

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FOREIGN PATENT DOCUMENTS

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		Office ³	Number ⁴	Kind Code ⁵ (if known)				

Examiner Signature

Kelli Ben

Date Considered

10/23/02

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		Number	Kind Code ² (if known)			
Q		5,059,393	A	Quenin <i>et al.</i>	10-22-1991	
		5,081,038	A	Sugaya <i>et al.</i>	1-14-1992	
		5,102,624	A	Muraishi	4-7-1992	
		5,106,583	A	Raysberg <i>et al.</i>	4-21-1992	
		5,250,262	A	Heidt <i>et al.</i>	10-5-1993	
		5,316,728	A	Hayashi <i>et al.</i>	5-31-1994	
		5,355,695	A	Kawaguchi <i>et al.</i>	10-18-1994	

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Examiner Signature	<i>Kyle Box</i>	Date Considered	10/24/02
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Substitute for form 1449A/PTO <div style="text-align: center;"> INFORMATION DISCLOSURE STATEMENT BY APPLICANT </div> (use as many sheets as necessary)				<i>Complete if Known</i>	
				Application No.	10/054,535
				Filing Date:	January 22, 2002
				First Named Inventor	Copeland
				Group Art Unit	3612 1793
				Examiner Name	unassigned Ben
				Attorney Docket No.	97,008-X
Sheet	5	of	5		

[illegible]

Examiner Signature	<i>Kathleen [Signature]</i>	Date Considered	10/25/02
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¹ Unique citation designation number. ² Applicant is to place a check mark here if English translation is attached.

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13



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/054,535	01/22/2002	Keith G. Copeland	97,008-X	7198

20306 7590 07/29/2003

MCDONNELL BOEHNEN HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
SUITE 3200
CHICAGO, IL 60606

EXAMINER

WARDEN, JILL ALICE

ART UNIT

PAPER NUMBER

1743

DATE MAILED: 07/29/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

10/054,535

Applicant(s)

COPELAND ET AL.

Examiner

Jill A. Warden

Art Unit

1743

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 06 May 2003.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 72-98 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 72-98 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- 11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.
- If approved, corrected drawings are required in reply to this Office action.
- 12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

- 13) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.
- 14) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).
- a) ☐ The translation of the foreign language provisional application has been received.
- 15) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) Paper No(s). _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO-1449) Paper No(s) <u>Z</u> . | 6) <input type="checkbox"/> Other: _____ |

Application/Control Number: 10/054,535
Art Unit: 1743

Page 2

DETAILED ACTION

The information disclosure statement (IDS) submitted on March 24, 2003 was filed after the mailing date of the first Office action on October 30, 2002. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Rejections - 35 USC § 103

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

Application/Control Number: 10/054,535

Page 3

Art Unit: 1743

2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 72-87 are rejected under 35 U.S.C. 103(a) as being unpatentable over Minekane in view of.

Minekane teaches an automatic chemical analysis apparatus including a plurality of separate reagents which are used in a plurality of different analysis reactions. The apparatus employs a reaction carousel which includes an annular of reaction cuvettes on the outer circumference and a plurality of reagent bottles inside the annular array. The apparatus includes a master control unit which includes information concerning each sample and different chemical tests to be performed on each sample as well as information on the content and location of the reagent bottles, which information has been input from "suitable data input means." Column 2, lines 19-34. Minekane specifically teaches that information on position and content of the reagent bottles is provided on bar codes affixed to the reagent bottles. Column 3, lines 60-65.

Conventional reagent dispensers transfer reagent from the reagent bottles to the reaction cuvettes, and a sample dispenser can be employed to dispense sample into the reaction cuvettes.

Minekane does not teach employing slides instead of cuvettes, and employing barcodes on the slides.

The use of sample slides instead of reaction cuvettes is well known in the art. See, for example Azuma, et al. who teach employing chemical analysis elements (11) (i.e. sample slides) in an automated chemical analyzer. Azuma, et al. also teach that

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Art Unit: 1743

the elements include a barcode (11a) which includes information on the type of test being performed on the slide. Column 3, lines 7-35. Azuma, et al. further teach that it is more conventional to use slides when employing dry chemical reagents. However, it is clearly appropriate to employ slides instead of cuvettes in order to lessen the amount of sample and reagents employed in the test.

It would have been obvious to one having ordinary skill in the art, to modify the apparatus of Minekane to employ slides with bar codes in place of the reaction cuvettes in order to minimize sample size, as well as employ dry chemistries. Such use of dry chemistries would not eliminate the use of reagent bottles, as other liquids, such as sample and wash liquids would still need to be provided to the slides. With respect to the barcodes, these would be considered "suitable data input means" as discussed in Minekane.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Application/Control Number: 10/054,535
Art Unit: 1743

Page 5


Claims 72-98 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-25 of U.S. Patent No. 6,352,861. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant claims are broader in scope than those of U.S. Patent No. 6,352,861, as the patent claims include limitations specific to reading the bar code to determine dispensing requirements. As such, the patent claims anticipate the application claims. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993).

Response to Arguments

Applicant's arguments, see paper no. 9, filed May 6, 2003, with respect to the rejection of claims over Heidt et al. in view of Sakurada et al. have been fully considered and are persuasive. The rejection of claims 72-98 over these references has been withdrawn. However, a new rejection based on references filed with the IDS of March 24, 2003, as well as a rejection over applicants' patent, U.S. 6,352,861 have been entered.

Conclusion

Any inquiry concerning this communication should be directed to Jill A. Warden at telephone number (703) 308-4037.


Jill Warden
Supervisory Patent Examiner
Technology Center 1700

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				Filing Date:	January 22, 2002
				First Named Inventor	Copeland
				Group Art Unit	1743
				Examiner Name	Patricia K. Bex
				Attorney Docket No.	97-008-X
Sheet	1	of	5		

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		Number	Kind Code ² (if known)			
JUN		3,482,082		Isreeli	12-2-1969	
		3,644,715		Holderith	2-22-1972	
		3,660,638		Oberli	5-2-1972	
		3,831,006		Chaffin, III et al.	8-20-1974	
		3,909,203		Young et al.	9-30-1975	
		3,916,157		Roulette et al.	10-28-1975	
		4,066,412		Johnson et al.	1-3-1978	
		4,133,642		Nosaka et al.	1-9-1979	
		4,135,883		McNeil et al.	1-23-1979	
JUN		4,159,875		Hauser	7-3-1979	

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		Office ³	Number ⁴	Kind Code ⁵ (if known)				
JUN ↓		JP	✓ 54014287 55107957		Agency of Ind Science & Technol	8-19-1980		X
		JP	✓ 55090892		Olympus Optical Co. Ltd.	1-27-1982		X
		JP	✓ 59189590		Hitachi Ltd.	4-8-1986		X
		WO	✓ 86/02163		American Hospital Supply Corporation	4-10-1986		
		JP	✓ 59216246		Hitachi Ltd.	5-14-1986		X
JUN		JP	✓ 60090544		Nippon Tectron Co. Ltd.	11-5-1986		X
Examiner Signature		JUN Warden				Date Considered	7/26/03	

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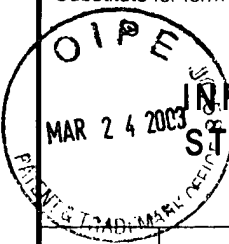
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		Number	Kind Code ² (if known)			
JW		4,163,643		Hunter et al.	8-7-1979	RECEIVED MAR 24 2003 TIC 1700
		4,281,387		Kraft et al.	7-28-1981	
		4,338,279		Orimo et al.	7-6-1982	
		4,371,498		Scordato et al.	2-1-1983	
		4,517,160		Galle et al.	5-14-1985	
		4,558,946		Galle et al.	12-17-1985	
		4,634,576		Galle et al.	1-6-1987	
		4,643,879		Hanaway	2-17-1987	
		4,647,432		Wakatake	3-3-1987	
JW		4,675,299		Witty et al.	6-23-1987	

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		JP	60116425		Hitachi Ltd.	12-5-1986		X
		JP	61035042		Toshiba Corp.	8-26-1987		X
		JP	61076122		Nippon Tectron Co. Ltd.	10-13-1987		X
		JP	61146064		Toshiba Corp.	1-8-1988		X
JW		JP	61190061		Hitachi Ltd.	2-26-1988		X
Examiner Signature		JWarden				Date Considered	7/26/03	

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Substitute for form 1449A/PTO

Complete if Known**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**

(use as many sheets as necessary)

Sheet	3	of	5	Application No.	10/054,535
				Filing Date	January 22, 2002
				First Named Inventor	Copeland
				Group Art Unit	1743
				Examiner Name	Patricia K. Bex
				Attorney Docket No.	97,008-X

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No. ¹	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines Where Relevant Passages or Figures Appear
		Number	Kind Code ² (if known)			
JAN		4,678,894		✓ Shafer	7-7-1987	
		4,683,120		✓ Meserol et al.	7-28-1987	
		4,692,308		✓ Riley et al.	9-8-1987	
		4,719,087		✓ Hanaway	1-12-1988	
		4,727,033		✓ Hijikata et al.	2-23-1988	
		4,795,613		✓ Azuma et al.	1-3-1989	
		4,808,380		✓ Minekane	2-28-1989	
		4,824,641		✓ Williams	4-25-1989	
		4,844,887		✓ Galle et al.	7-4-1989	
JAN		4,847,208		✓ Bogen	7-11-1989	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No. ¹	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines Where Relevant Passages or Figures Appear	T ⁶
		Office ³	Number ⁴	Kind Code ⁵ (if known)				
JAN		JP	✓ 61205089		Toshiba Corp.	3-17-1988		X
		WO	✓ 88/02866		Serono Diagnostics Partners	4-21-1988		
		JP	✓ 61242989		Hitachi Ltd.	4-30-1988		X
		JP	✓ 61275282		Nittec Co. Ltd.	6-3-1988		X
		EP	✓ 0285851	A2	Fuji Photo Film Co., Ltd.	10-12-1988		
		EP	✓ 0290018	A2	Abbott Laboratories	11-9-1988		
JAN		JP	✓ 62202748		Toshiba Corp.	2-17-1989		X
Examiner Signature		J. Warden				Date Considered	7/26/03	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ Unique citation designation number. ² Applicant is to place a check mark here if English translation is attached.

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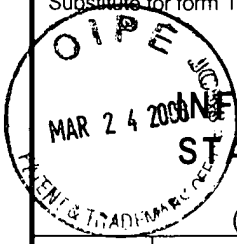
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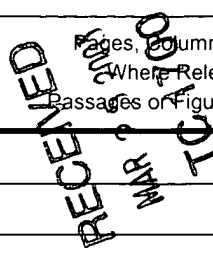
PTO/SB/08B (08-00)

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Substitute for form 1449A/PTO				Complete if Known	
 INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)				Application No.	10/054,535
				Filing Date:	January 22, 2002
				First Named Inventor	Copeland
				Group Art Unit	1743
				Examiner Name	Patricia K. Bex
Sheet	4	of	5	Attorney Docket No.	97,008-X

U.S. PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines Where Relevant Passages or Figures Appear
		Number	Kind Code ² (if known)			
JWW		4,855,110		✓ Marker et al.	8-8-1989	
		5,031,797		✓ Boris et al.	7-16-1991	
		5,073,504		✓ Bogen	12-17-1991	
		5,311,426		✓ Donohue et al.	5-10-1994	
		5,316,452		✓ Bogen et al.	5-31-1994	
JWW		5,418,138		✓ Miller et al.	5-23-1995	

FOREIGN PATENT DOCUMENTS								
Examiner Initials*	Cite No. ¹	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines Where Relevant Passages or Figures Appear	T ⁶
		Office ³	Number ⁴	Kind Code ⁵ (if known)				
JWW		WO	89/01616		Arthur Harris	2-23-1989		
		JP	63082232		Toshiba Corp.	10-11-1989		X
		JP	63144871		Toshiba Corp.	12-19-1989		X
Examiner Signature	JWWarden					Date Considered	7/26/03	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ Unique citation designation number. ² Applicant is to place a check mark here if English translation is attached.

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Substitute for form 1449A/PTO

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**

(use as many sheets as necessary)

Sheet

5

of

5

Application No.

10/054,535

Filing Date

January 22, 2002

First Named Inventor

Copeland

Group Art Unit

1743

Examiner Name

Patricia K. Bex

Attorney Docket No.

97,008-X

OTHER DOCUMENTS -- NON PATENT LITERATURE DOCUMENTSExaminer
Initials*Cite
No.
1

Include name of author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published

T²

DRISCOLL et al., "Discrete Automated Chemistry System with Tableted Reagents," Clinical Chemistry, 29(9):1609-1615, 1983.

RAPPOPORT, "If Bar Code Works in Supermarkets, It Should Be Great for Medicine," Pathologist, 39(2):39-40, 1985.

BECKMAN INSTRUMENTS, "Premarket Notifications (Section 510(k) Notices) for Synchron CX 4 and Synchron CX 5 Clinical Chemistry Analyzers," April 5, 1988.

TILZER et al., "Use of Bar Code Labels on Collection Tubes for Specimen Management in the Clinical Laboratory," Arch Pathol Lab Med, 112:1201-1202, Dec. 1988.

CHOW et al., "Application of Existing Technology to Meet Increasing Demands for Automated Sample Handling," Clinical Chemistry, 36(9):1579-1582, 1990.

GARZA et al., "Bar Codes in the Clinical Laboratory," Clinical Laboratory Science, 4(1):23-24, Jan/Feb 1991.

Examiner
Signature*J. Warden*Date
Considered

7/26/03

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ Unique citation designation number. ² Applicant is to place a check mark here if English translation is attached.

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EXHIBIT

14



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/054,535	01/22/2002	Keith G. Copeland	97,008-X	7198
20306	7590	04/22/2004	EXAMINER	
MCDONNELL BOEHNEN HULBERT & BERGHOFF LLP			WARDEN, JILL ALICE	
300 S. WACKER DRIVE			ART UNIT	PAPER NUMBER
32ND FLOOR			1743	
CHICAGO, IL 60606			DATE MAILED: 04/22/2004	

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 10/054,535	Applicant(s) COPELAND ET AL.	
	Examiner Jill A. Warden	Art Unit 1743	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 02 February 2004.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 72-98 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☒ Claim(s) 92-98 is/are allowed.
- 6) ☒ Claim(s) 72-74 and 79-87 is/are rejected.
- 7) ☒ Claim(s) 75-77 and 88-91 is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

Application/Control Number: 10/054,535
Art Unit: 1743

Page 2

DETAILED ACTION

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 72-74 and 79-87 are rejected under 35 U.S.C. 103(a) as being unpatentable over Minekane in view of Azuma, et al.

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Page 3

Minekane teaches an automatic chemical analysis apparatus including a plurality of separate reagents which are used in a plurality of different analysis reactions. The apparatus employs a reaction carousel which includes an annular array of reaction cuvettes on the outer circumference and a plurality of reagent bottles inside the annular array. The apparatus includes a master control unit which includes information concerning each sample and different chemical tests to be performed on each sample as well as information on the content and location of the reagent bottles, which information has been input from "suitable data input means." Column 2, lines 19-34. Minekane specifically teaches that information on position and content of the reagent bottles is provided on bar codes affixed to the reagent bottles. Column 3, lines 60-65. Conventional reagent dispensers transfer reagent from the reagent bottles to the reaction cuvettes, and a sample dispenser can be employed to dispense sample into the reaction cuvettes.

Minekane does not teach employing slides instead of cuvettes, and employing barcodes on the slides.

The use of sample slides instead of reaction cuvettes is well known in the art. See, for example Azuma, et al. who teach employing chemical analysis elements (11) (i.e. sample slides) in an automated chemical analyzer. Azuma, et al. also teach that the elements include a barcode (11a) which includes information on the type of test being performed on the slide. Column 3, lines 7-35. Azuma, et al. further teach that it is more conventional to use slides when employing dry chemical reagents. However, it is

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Art Unit: 1743

clearly appropriate to employ slides instead of cuvettes in order to lessen the amount of sample and reagents employed in the test.

It would have been obvious to one having ordinary skill in the art, to modify the apparatus of Minekane to employ slides with bar codes in place of the reaction cuvettes in order to minimize sample size, as well as employ dry chemistries. Such use of dry chemistries would not eliminate the use of reagent bottles, as other liquids, such as sample and wash liquids would still need to be provided to the slides. With respect to the barcodes, these would be considered "suitable data input means" as discussed in Minekane.

Terminal Disclaimer

The terminal disclaimer filed on February 2, 2004 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of U.S. patent no. 6,352,861 has been reviewed and is accepted. The terminal disclaimer has been recorded.

Allowable Subject Matter

Claims 92-98 are allowed.

Claims 75-78 and 88-91 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

The following is a statement of reasons for the indication of allowable subject matter: The prior art does not teach, nor fairly suggest, reading identifying information from a bar code on the slide to determine whether or not reagent should be dispensed

Application/Control Number: 10/054,535
Art Unit: 1743

Page 5

onto the slide, relative movement between the slide support and reagent containers or mechanical actuation of the reagent containers within a method of dispensing reagents onto a slide substantially as claimed in the instant claims.

Response to Arguments

Applicant's arguments filed February 2, 2004 have been fully considered but they are not persuasive. Applicant argues there is no teaching to combine the references and that one of ordinary skill would not have been motivated to look to the secondary reference, as it is a very different method. Examiner disagrees. Both references are directed to automatic chemical analyzers, which are indeed, the same field of endeavor. As to suggestion for combining references, the examiner has supplied such. No such suggestion need come directly from the references. One of ordinary skill in the art has some knowledge of problems and solutions and would be so motivated to look to similar art to find solutions for problems in dispensing in chemical analyzers.

Applicant further argues that the combined teachings do not provide for dispensing on a slide, as Azuma teaches a measuring element, not a slide. Examiner takes the position that the measuring element of Azuma is a slide, as slide is understood to one of ordinary skill in the analytical chemistry art.

Applicant argues that neither reference determines if a reagent should be dispensed by using identifying information from the slide. Again, examiner disagrees. Position of the slide or cuvette is information from the slide or cuvette used to dispense reagents and sample. Both Minekane and Azuma teach reading positional information from the slide or cuvette.

Application/Control Number: 10/054,535
Art Unit: 1743

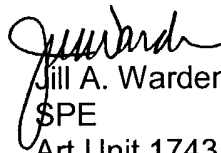
Page 6

Conclusion

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication should be directed to Jill A. Warden at telephone number (571) 272-1267.


Jill A. Warden
SPE
Art Unit 1743

EXHIBIT

15

Rejected Claim 73 from the prosecution of US Patent 6,943,029

72. A method of dispensing reagents onto a slide, the method comprising the steps of:
providing at least one reagent container;
providing at least one slide on a slide support;
automatically identifying the reagent container using a computer, the step of automatically
identifying being based on information associated with the reagent container;
automatically determining whether reagent in the reagent container should be dispensed onto
the slide; and

dispensing the reagent in the reagent container onto the slide based on the determination of
whether the reagent in the reagent container should be dispensed onto the slide.

73. The method of claim 72, wherein the step of automatically determining whether
reagent in the reagent container should be dispensed onto the slide includes identifying information
from the slide.

Claim 1 of US Patent 6,943,029 as allowed

What is claimed is:

1. A method of dispensing reagents onto a slide, the
method comprising the steps of:
50 providing at least one reagent container;
providing at least one slide on a slide support;
automatically identifying the reagent container using a
computer, the step of automatically identifying being
based on information associated with the reagent
55 container;
automatically determining whether reagent in the reagent
container should be dispensed onto the slide; and
dispensing the reagent in the reagent container onto the
60 slide based on the determination of whether the reagent
in the reagent container should be dispensed onto the
slide wherein the step of automatically determining
whether reagent in the reagent container should be
dispensed onto the slide includes identifying barcode
65 information from the slide.

EXHIBIT

16

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 97,008-X1)

In re Application of:)	
)	
COPELAND, et. al)	
)	Group Art Unit: 1743
Serial No.: Unknown)	
)	Examiner: Brian Gordon
Filed: November 17, 2004)	
)	
For: Automated Biological)	
Reaction Apparatus)	

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Dear Sir:

Please enter this Preliminary Amendment before calculating the filing fee for this application.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the **Listing of the Claims** which begins on page 4 of this paper.

Remarks begin on page 14 of this paper.

IN THE SPECIFICATION:

Please amend the paragraph beginning at page 1, line 3-10 as follows:

This is a continuation of co-pending application Serial No. 10/054535, filed on January 22, 2002 which is a continuation of application Serial No. 09/452,309, filed December 1, 1999, now U.S. Patent No. 6,352,861, which is a continuation of application Serial No. 08/906,678, filed August 5, 1997, now abandoned, which is a continuation of application Serial No. 08/479,415, filed June 6, 1995, now U.S. Patent No. 5,654,200, which is a division of application Serial No. 08/352,966, filed December 9, 1994, now U.S. Patent No. 5,595,707, which is a continuation of application Serial No. 07/924,052, filed August 31, 1992, now abandoned, which is a Rule 371 application of PCT/US91/01149 filed on February 28, 1991, which in turn claims benefit to application Serial No. 07/488,601, filed March 2, 1990, now abandoned.

Please insert the following paragraph after the paragraph ending on page 3, line 24 and before the paragraph that begins on page 3, line 25:

Optically encoded identifiers, such as bar code identifiers, are often used to provide information about an article, and in particular, a moving article to which a bar code is associated. Bar code optical identifiers come in many shapes, forms and designs. For example it is well known that bar codes exist in one dimensional and in multi-dimensional forms (e.g. two dimensional). The primary difference between one and multi-dimensional bar codes lies in the amount of information carried by the bar code with multi-dimensional bar codes, such as those disclosed in U.S. Patent No. 5,591,956, able to convey more information than one dimensional bar codes. Moreover, devices for reading bar codes, including multi-dimensional barcodes are well known in the art. An example of such a device capable of reading bar codes is disclosed in U.S. Patent No. 5,235,167.

Following page 12, line 10, please insert the following paragraph:

FIG. 34 is a schematic of a jet drain for draining liquid from an upper surface of a slide.

Please amend the paragraph at page 10, lines 16 - 18 as follows:

Figs. 18A –C ~~is a~~are schematic representational cross-sectional views of a slide following the rinse liquid (Fig. 18A), evaporation inhibitor (Fig 18B) and reagent application (Fig. 18C) steps.

Please amend the paragraph at page 41, lines 3-10 as follows:

Immunohistological methods for which the apparatus of this invention are particularly suitable described in concurrently filed, commonly assigned patent application serial no. 07/488,601, filed March 2, 1990 now abandoned (~~attorney Docket No. 193.0007~~). The entire contents of which are hereby incorporated by reference. A typical immunohistological method, as carried with apparatus of this invention includes the following steps:

IN THE CLAIMS:

This listing of claims will replace all prior versions, and listing, of claims in the application.

Listing Of The Claims:

1-71 (Cancelled)

72. (New) An automated method of dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container;

providing at least one slide on a slide support;

automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container;

automatically determining whether reagent from the reagent container should be dispensed onto the slide; and

automatically dispensing the reagent onto the slide based on the determination of whether the reagent should be dispensed onto the slide, wherein the step of automatically determining whether reagent should be dispensed onto the slide includes acquiring information from an optically-encoded identifier associated with the slide.

73. (new) The automated method of claim 72 wherein said information associated with the reagent container comprises at least one optically-encoded identifier.

74. (new) The automated method of claim 73 wherein said at least one reagent container optically-encoded identifier comprises a bar code.

75. (new) The automated method of claim 72 wherein said optically-encoded identifier associated with the slide comprises a bar code.

76. (new) The automated method of claim 73 wherein the step of automatically identifying the reagent container comprises reading the reagent container optically-encoded identifier to identify the reagent.

77. (new) The automated method of claim 76 wherein the reagent container optically-encoded identifier comprises a bar code.

78. (new) The automated method of claim 72 wherein the step of automatically determining whether reagent from the reagent container should be dispensed onto the slide comprises using an optically-encoded identifier to identify a staining protocol to be applied to the slide.

79. (new) The automated method of claim 78 wherein said optically-encoded identifier comprises a bar code.

80. (new) The method of claim 78 wherein said staining protocol information is located remote from said optically-encoded identifier.

81. (New) A method of automatically dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container containing a reagent, said reagent container also having optically-encoded information associated with it;

providing at least one slide on a slide support, said slide having optically-encoded information associated with it;

automatically identifying said reagent container using a computer, the step of automatically identifying including reading said reagent container optically-encoded information; and

automatically determining whether said reagent from said reagent container should be dispensed onto said slide based on reading said optically-encoded information associated with said slide.

82. (new) The automated method of claim 81 wherein said optically-encoded information associated with the reagent container comprises at least one optical identifier.

83. (new) The automated method of claim 82 wherein said at least one optical identifier comprises bar code-encoded information.

84. (new) The automated method of claim 81 wherein said optically-encoded information from the at least one slide comprises at least one optical identifier.

85. (new) The automated method of claim 82 wherein the step of automatically identifying the at least one reagent container comprises reading the optical identifier associated with the reagent container to identify the reagent contained in the reagent container.

86. (new) The automated method of claim 85 wherein the optical identifier associated with the reagent container comprises bar code-encoded information.

87. (new) The automated method of claim 81 wherein the step of automatically determining whether reagent from the at least one reagent container should be dispensed onto the at least one slide comprises using an optical identifier to identify a staining protocol to be applied to the slide.

88. (new) The automated method of claim 87 wherein said optical identifier to identify a staining protocol comprises bar code-encoded information.

89. (new) The method of claim 88 wherein said staining protocol information is located remote from said optical identifier.

90. (new) An automated method of dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container;

providing at least one slide on a slide support;

automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container; automatically determining whether the reagent from the reagent container should be dispensed onto the slide; and

automatically dispensing the reagent onto the slide based on the determination of whether the reagent should be dispensed onto the slide, wherein the step of automatically determining whether reagent should be dispensed onto the slide includes acquiring machine-readable information associated with the slide.

91. (new) The automated method of claim 90 wherein said information associated with the reagent container comprises at least one machine-readable identifier.

92. (new) The automated method of claim 91 wherein said at least one machine-readable identifier comprises an optical identifier.

93. (new) The automated method of claim 92 wherein said optical identifier comprises a bar code.

94. (new) The automated method of claim 91 wherein the step of automatically identifying the reagent container comprises reading the machine-readable identifier associated with the reagent container to identify the reagent.

95. (new) The automated method of claim 94 wherein the machine-readable identifier associated with the reagent container comprises optically-encoded information.

96. (new) The automated method of claim 90 wherein the step of automatically determining whether reagent from the reagent container should be dispensed onto the slide comprises using a machine-readable identifier to identify a staining protocol to be applied to the slide.

97. (new) The automated method of claim 96 wherein said machine-readable identifier comprises optically-encoded information.

98. (new) The method of claim 96 wherein said staining protocol information is located remote from said machine-readable identifier.

99. (new) The method of claim 98 wherein said machine-readable identifier comprises optically-encoded information.

100. (new) An automated method of dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container;

providing at least one slide on a slide support;

automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container;

automatically determining whether reagent in the reagent container should be dispensed onto the slide; and

automatically dispensing the reagent onto the slide based on the determination of whether the reagent should be dispensed onto the slide, wherein the step of automatically determining whether reagent should be dispensed onto the slide includes acquiring information from an optically-encoded symbol associated with the slide.

101. (new) The automated method of claim 100 wherein said information associated with the reagent container comprises at least one optically-encoded symbol.

102. (new) The automated method of claim 101 wherein said reagent container-associated optically-encoded symbol comprises a bar code.

103. (new) The automated method of claim 101 wherein the step of automatically identifying the reagent container comprises reading the optically-encoded symbol associated with the reagent container to identify the reagent.

104. (new) The automated method of claim 103 wherein the optically-encoded symbol comprises a bar code.

105. (new) The automated method of claim 100 wherein the step of automatically determining whether reagent from the reagent container should be dispensed onto the slide comprises using an optically-encoded symbol to identify a staining protocol to be applied to the slide.

106. (new) The automated method of claim 105 wherein said optically-encoded symbol comprises a bar code.

107. (new) The method of claim 105 wherein said staining protocol information is located remote from said optically-encoded symbol.

REMARKS

Claims 1-71 have been cancelled from this application. New claims 72-107 have been added to the application. The specification has been amended to update the application priority claim and to correct typographical errors in the specification. Moreover, the background of the invention has been updated to reflect the state of the art of barcodes and equivalent identifiers as of the earliest claimed filing date of the present application. No new matter has been added to the application by way of these specification claim amendments.

Respectfully submitted,

McDonnell Boehnen Hulbert & Berghoff

Date: November 17, 2004

By: 

A. Blair Hughes

Reg. No. 32,901

312-913-2123

EXHIBIT

17

(12) **United States Patent**
Copeland et al.

(10) **Patent No.:** **US 6,943,029 B2**
(45) **Date of Patent:** **Sep. 13, 2005**

(54) **AUTOMATED BIOLOGICAL REACTION APPARATUS**

(75) Inventors: **Keith G. Copeland**, Tucson, AZ (US);
Thomas M. Grogan, Tucson, AZ (US);
Charles Hassen, Tucson, AZ (US);
William Ross Humphreys, Tucson, AZ
(US); **Charles D. Lemme**, Tucson, AZ
(US); **Phillip C. Miller**, Tucson, AZ
(US); **William L. Richards**, Tucson,
AZ (US); **Wayne A. Showalter**,
Tucson, AZ (US)

(73) Assignee: **Ventana Medical Systems, Inc.**,
Tucson, AZ (US)

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patent is extended or adjusted under 35
U.S.C. 154(b) by 42 days.

(21) Appl. No.: **10/054,535**

(22) Filed: **Jan. 22, 2002**

(65) **Prior Publication Data**

US 2002/0072122 A1 Jun. 13, 2002

Related U.S. Application Data

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1999, now Pat. No. 6,352,861, which is a continuation of
application No. 08/906,678, filed on Aug. 5, 1997, now
abandoned, which is a continuation of application No.
08/479,415, filed on Jun. 6, 1995, now Pat. No. 5,654,200,
which is a division of application No. 08/352,966, filed on
Dec. 9, 1994, now Pat. No. 5,595,707, which is a continu-
ation of application No. 07/924,052, filed on Aug. 31, 1992,
now abandoned, which is a continuation-in-part of applica-
tion No. 07/488,601, filed on Mar. 2, 1990, now abandoned.

(51) **Int. Cl.**⁷ **G01N 35/00**; G01N 1/00;
G01N 1/10; B01L 3/02; B01L 9/00

(52) **U.S. Cl.** **436/46**; 436/43; 436/174;
436/180; 436/46; 422/63; 422/64; 422/67;
422/100

(58) **Field of Search** 436/43, 46, 174,
436/180; 422/63, 64, 100, 67, 104

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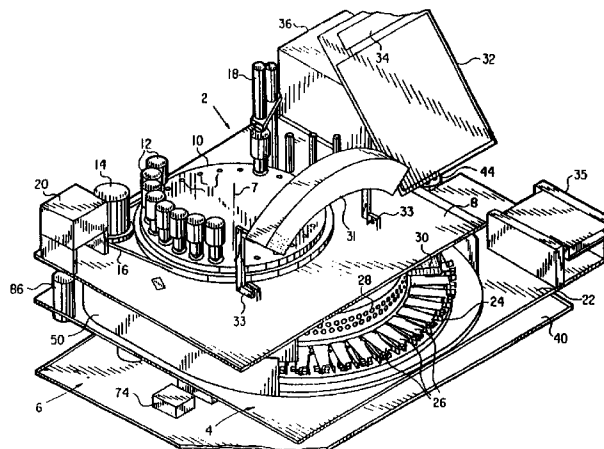
Primary Examiner—Jill Warden
Assistant Examiner—Brian R. Gordon

(74) *Attorney, Agent, or Firm*—McDonnell Boehnen
Hulbert & Berghoff LLP

(57) **ABSTRACT**

An automated immunostaining apparatus having a reagent
application zone and a reagent supply zone. The apparatus
has a carousel slide support supporting a plurality of slide
supports thereon, and drive means engaging the carousel
slide support for consecutively positioning each of a plural-
ity of slide supports in the reagent application zone. The
apparatus also has a carousel reagent support having a
plurality of reagent container supports thereon, and drive
means engaging the carousel for rotating the carousel and
positioning a preselected reagent container support in the
reagent supply zone. The apparatus also has a reagent
delivery actuator means positioned for engaging a reagent
container positioned on a container support in the reagent
delivery zone and initiating reagent delivery from the
reagent container to a slide supported on a slide support in
the reagent receiving zone.

24 Claims, 37 Drawing Sheets



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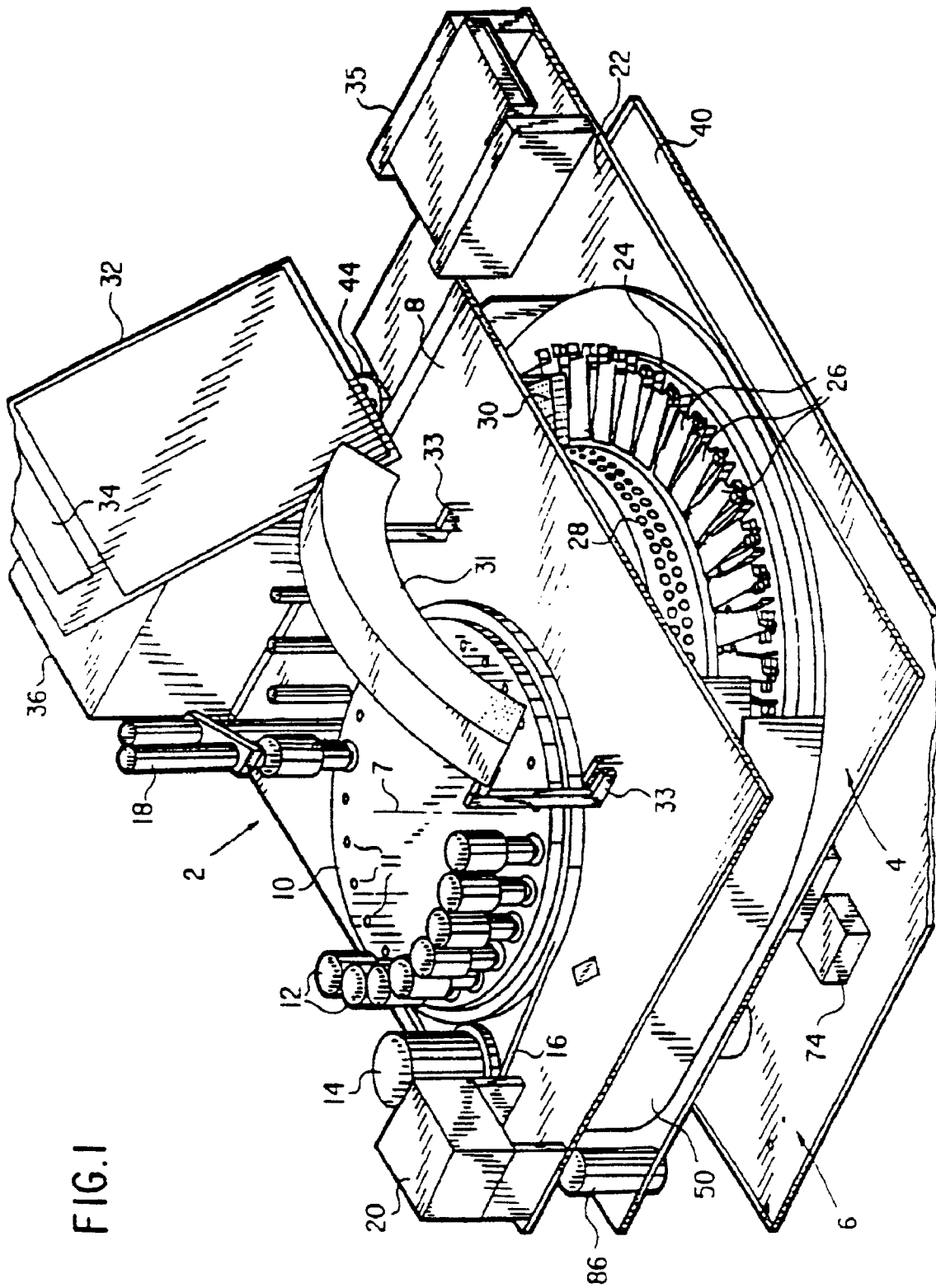
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FIG. 1



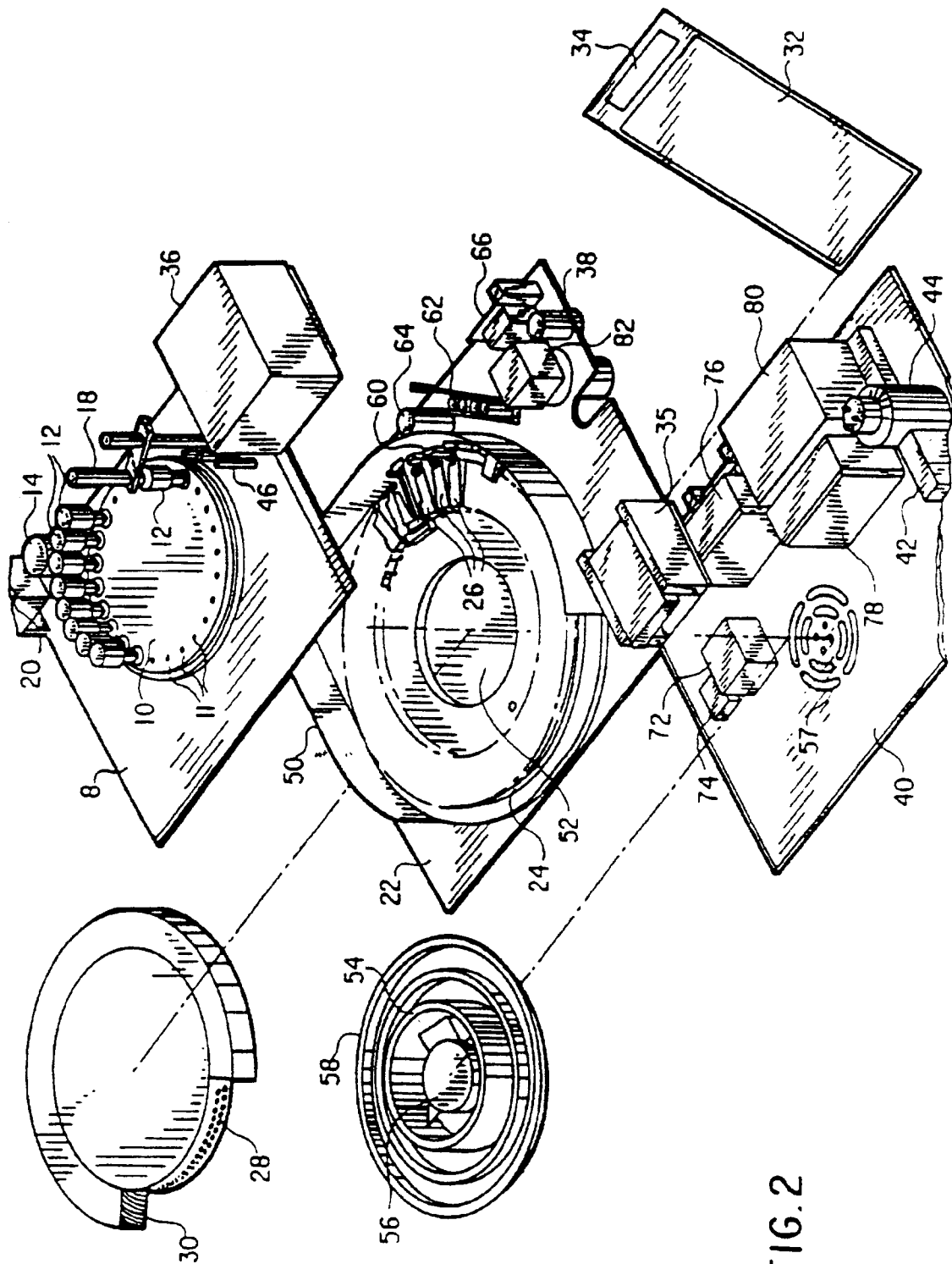


FIG. 2

FIG. 3

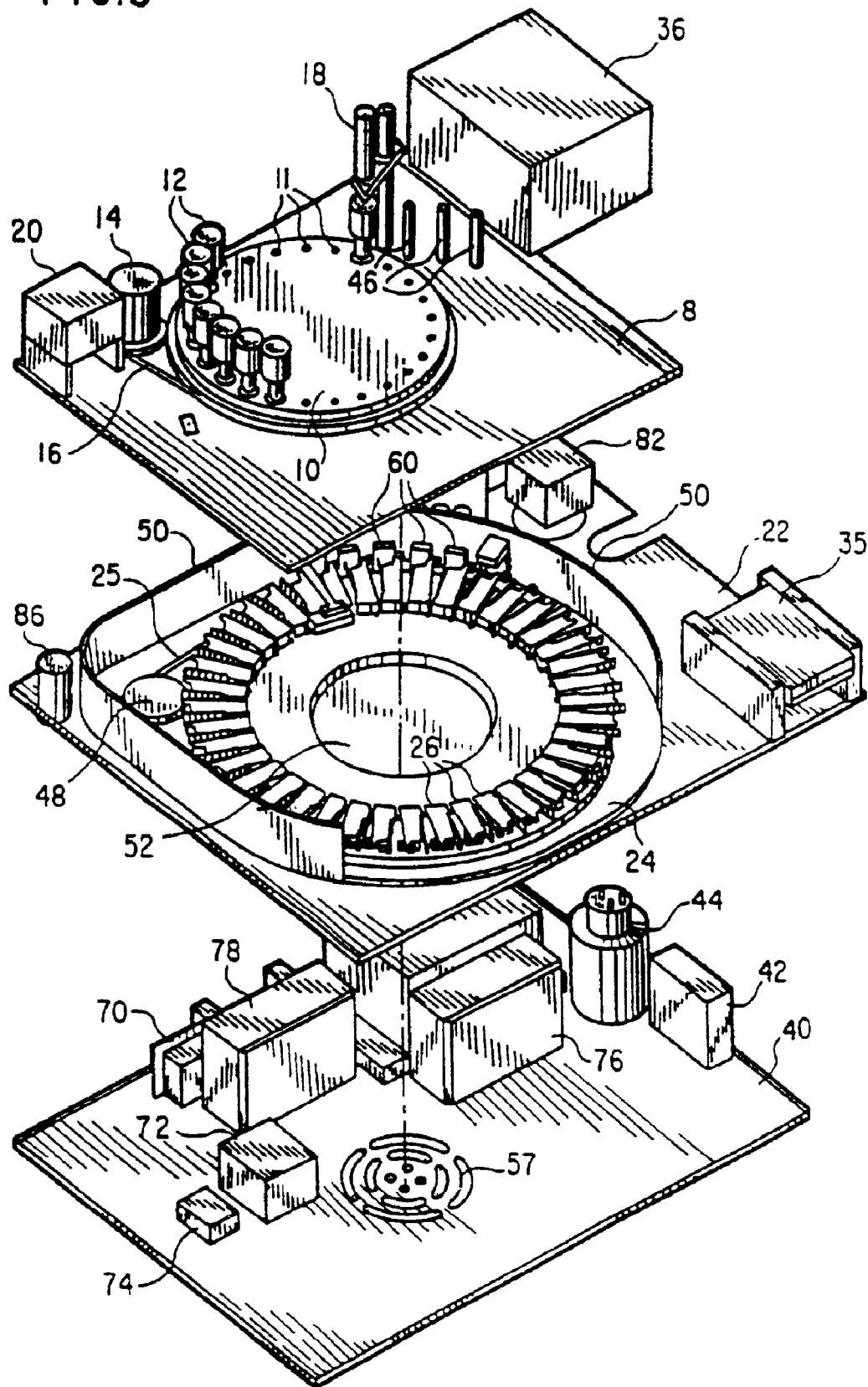
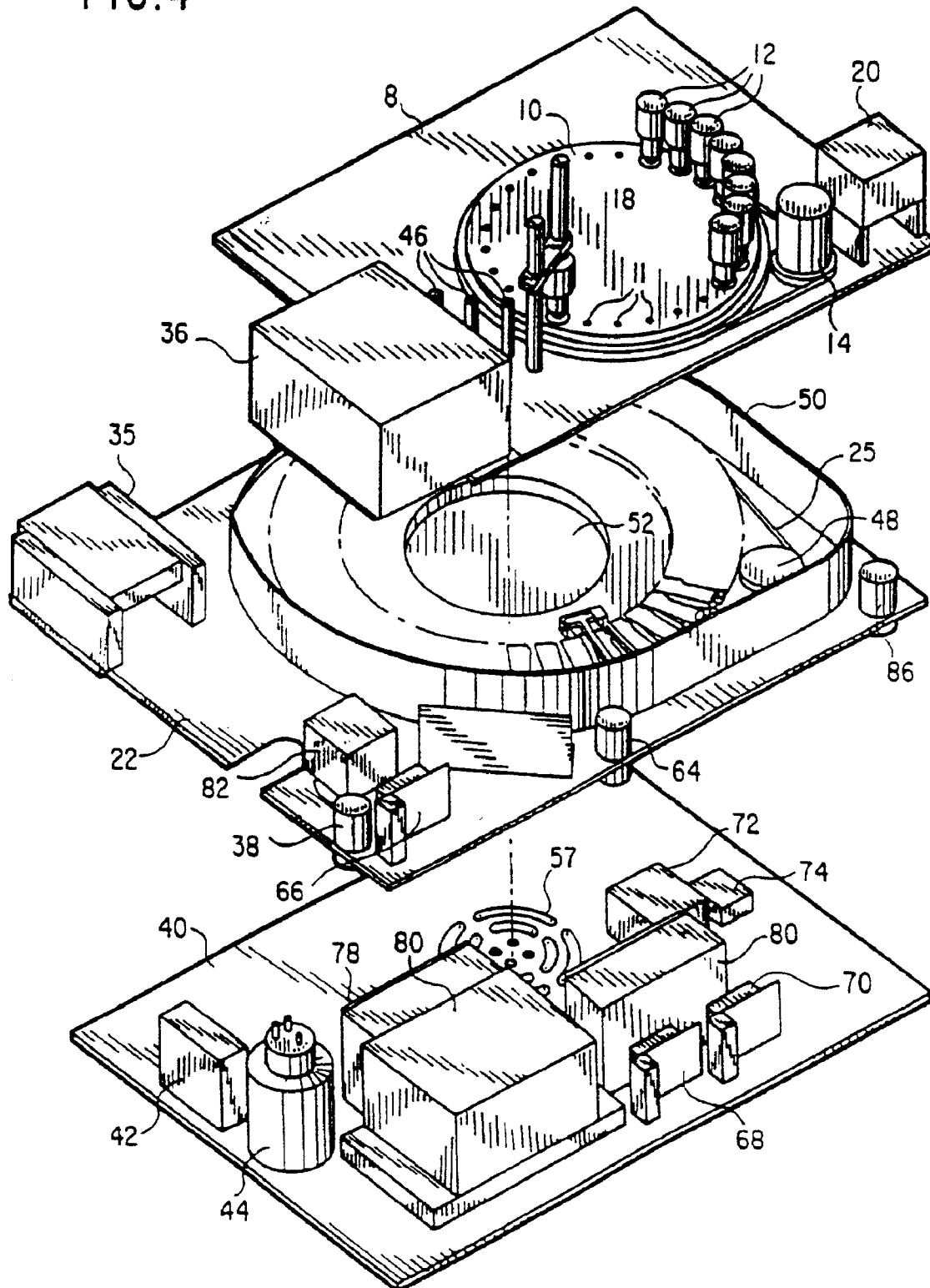


FIG. 4



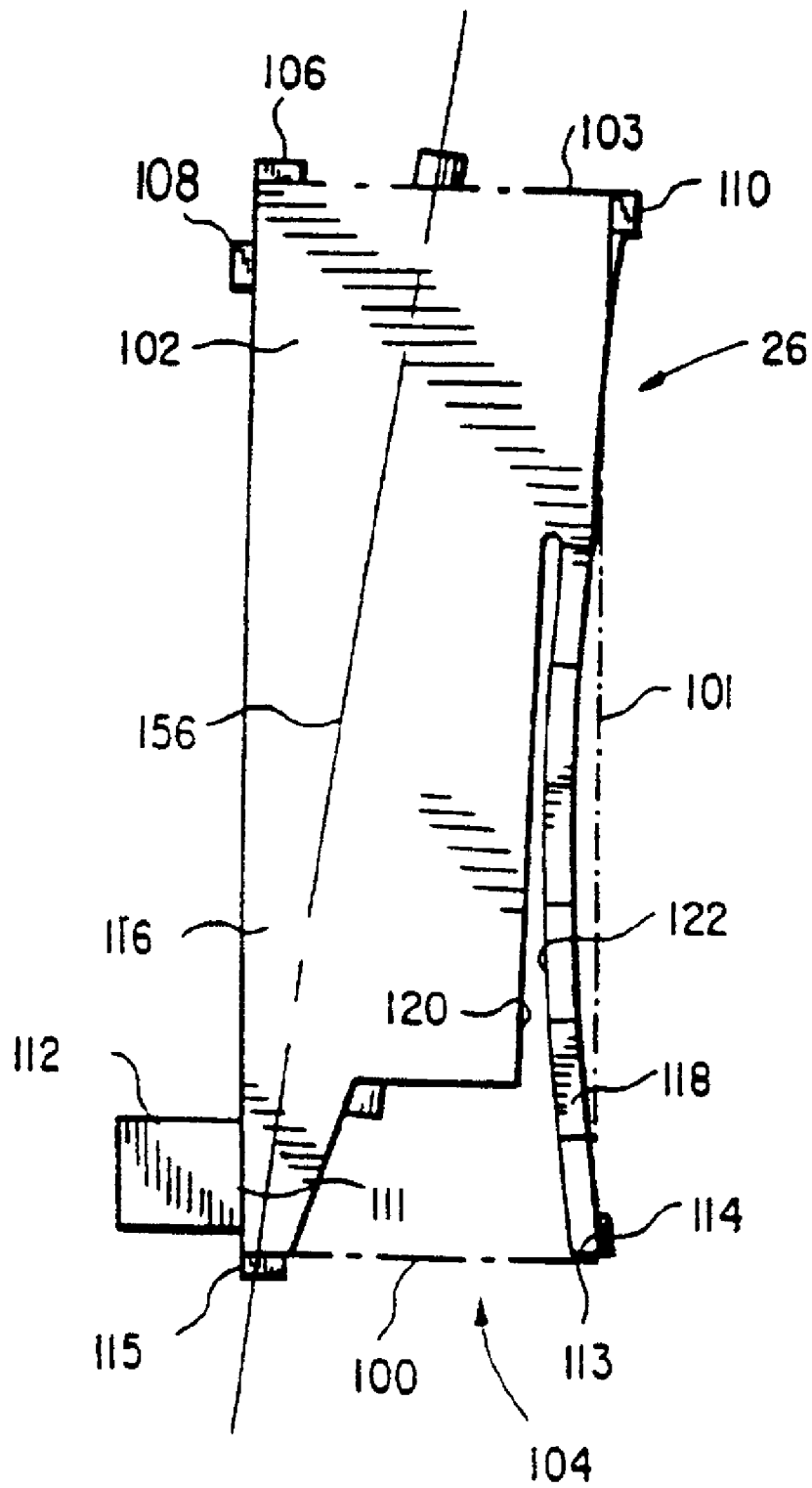


FIG. 5

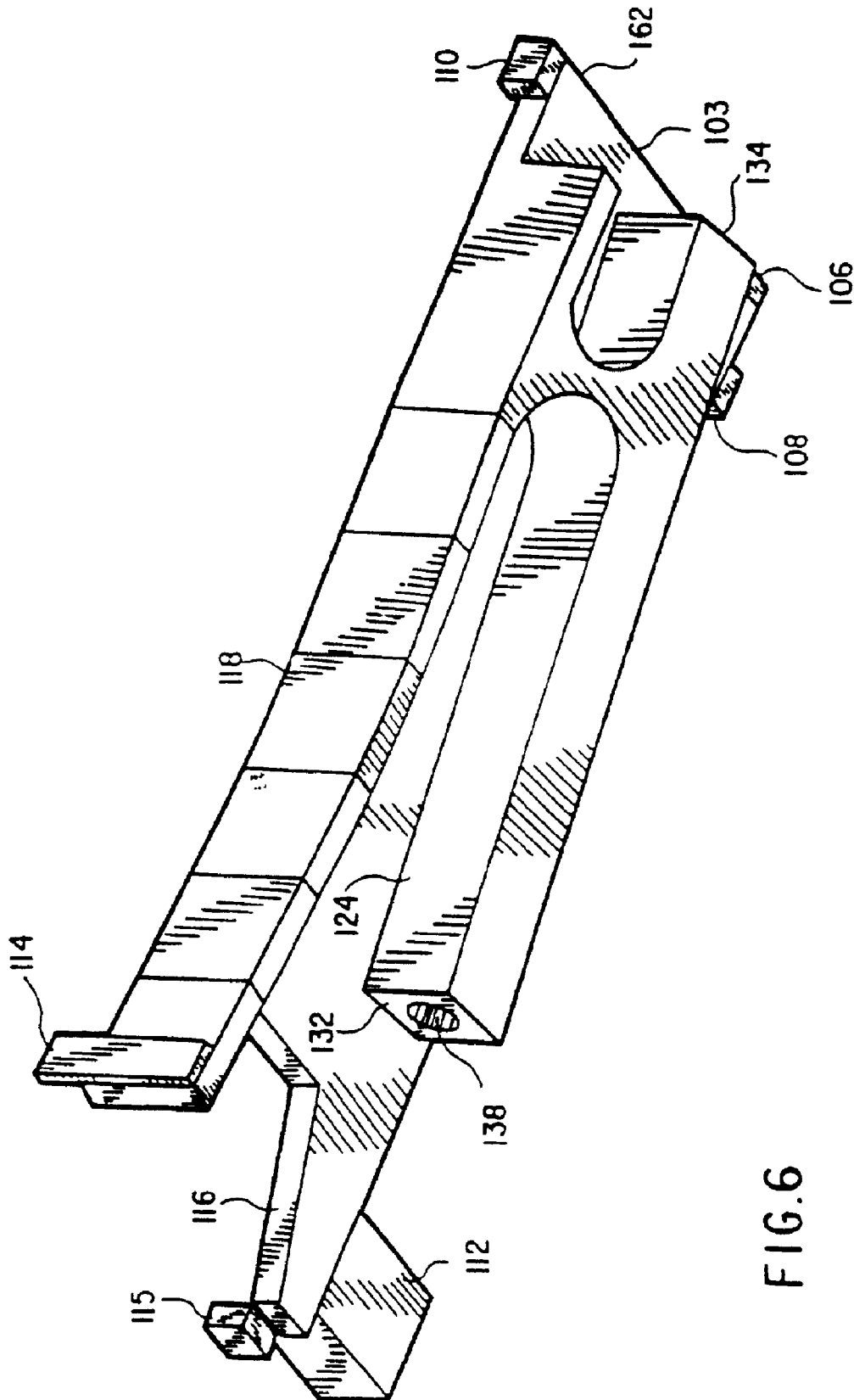


FIG. 6

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FIG. 7

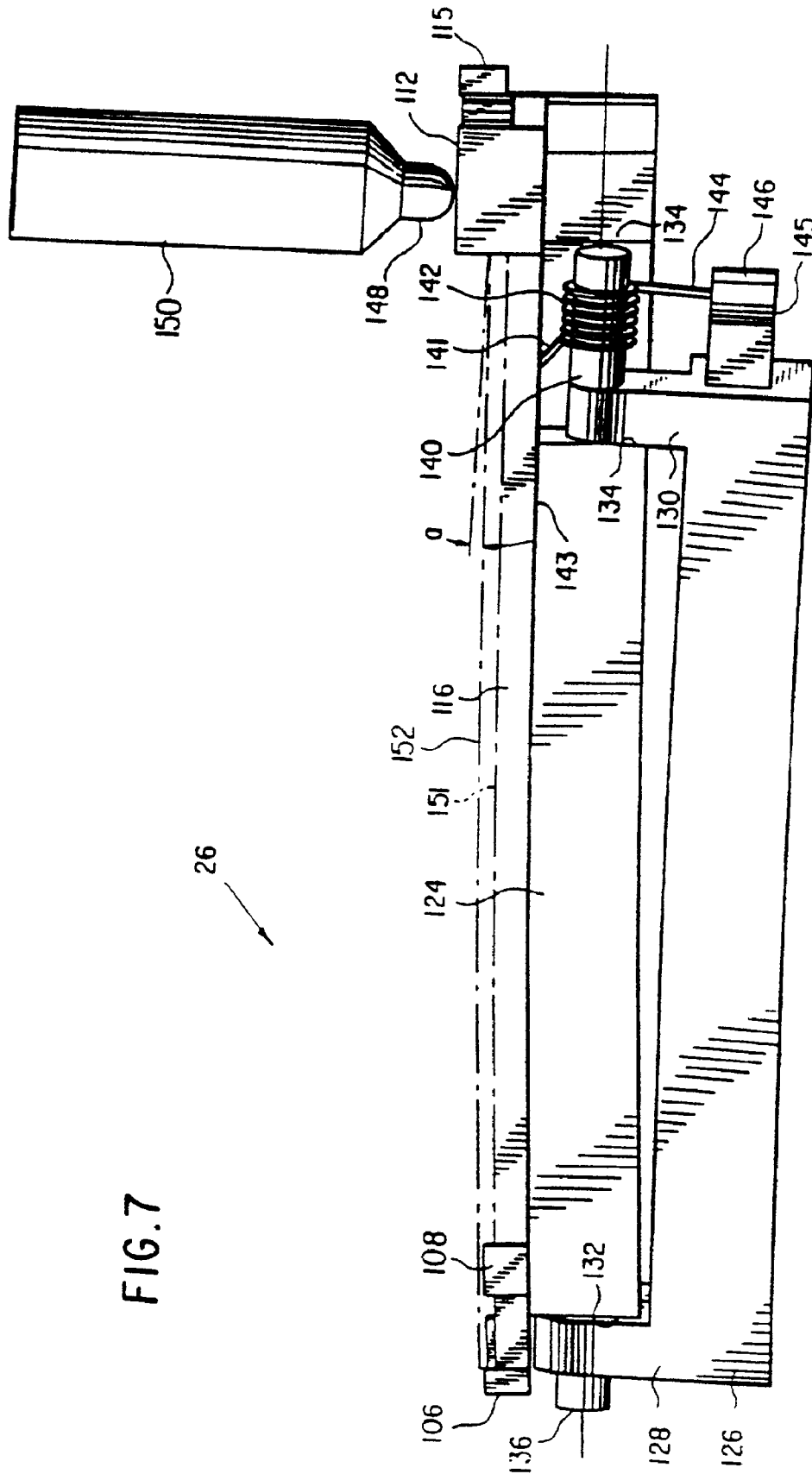


FIG. 8

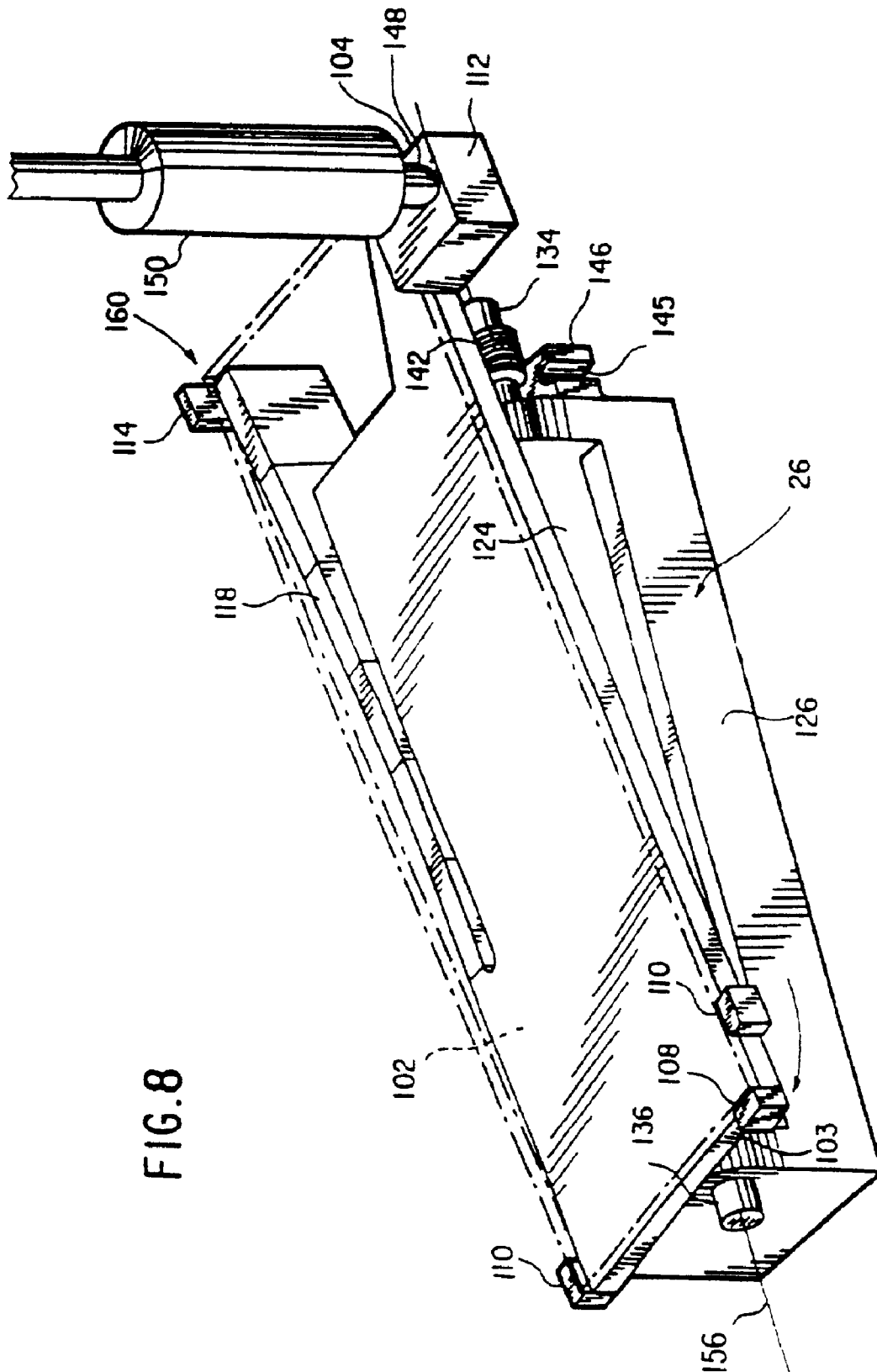


FIG. 9

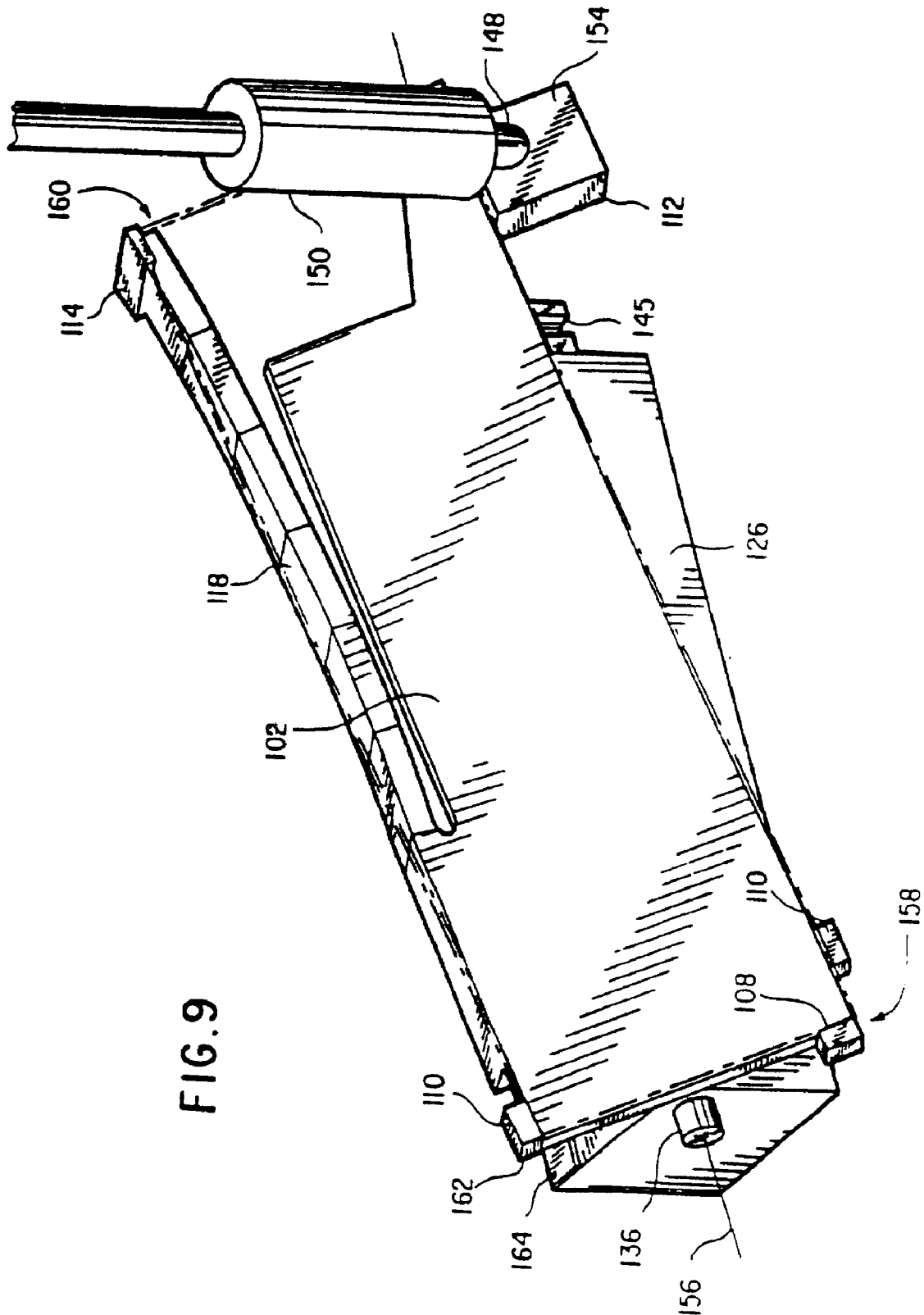
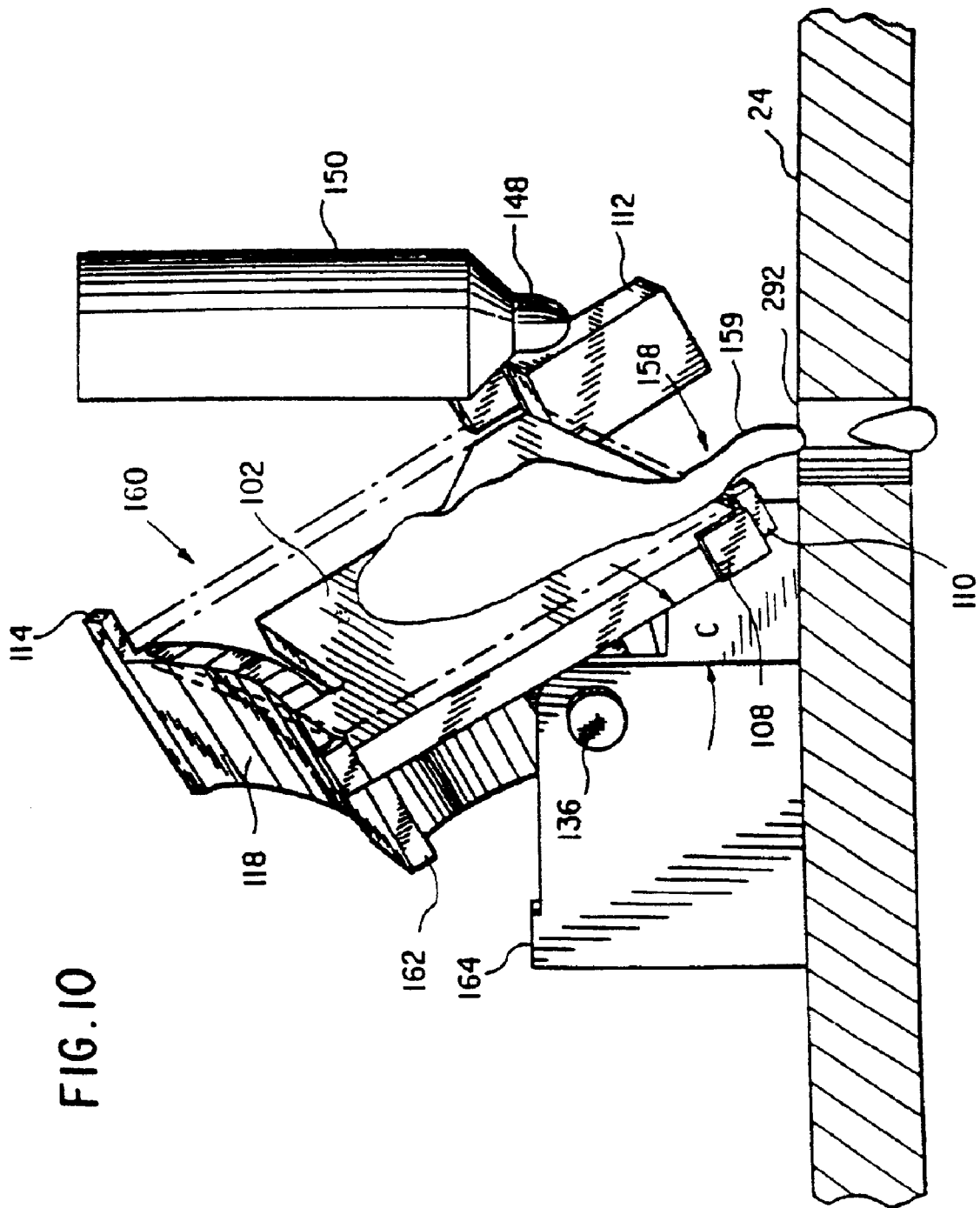
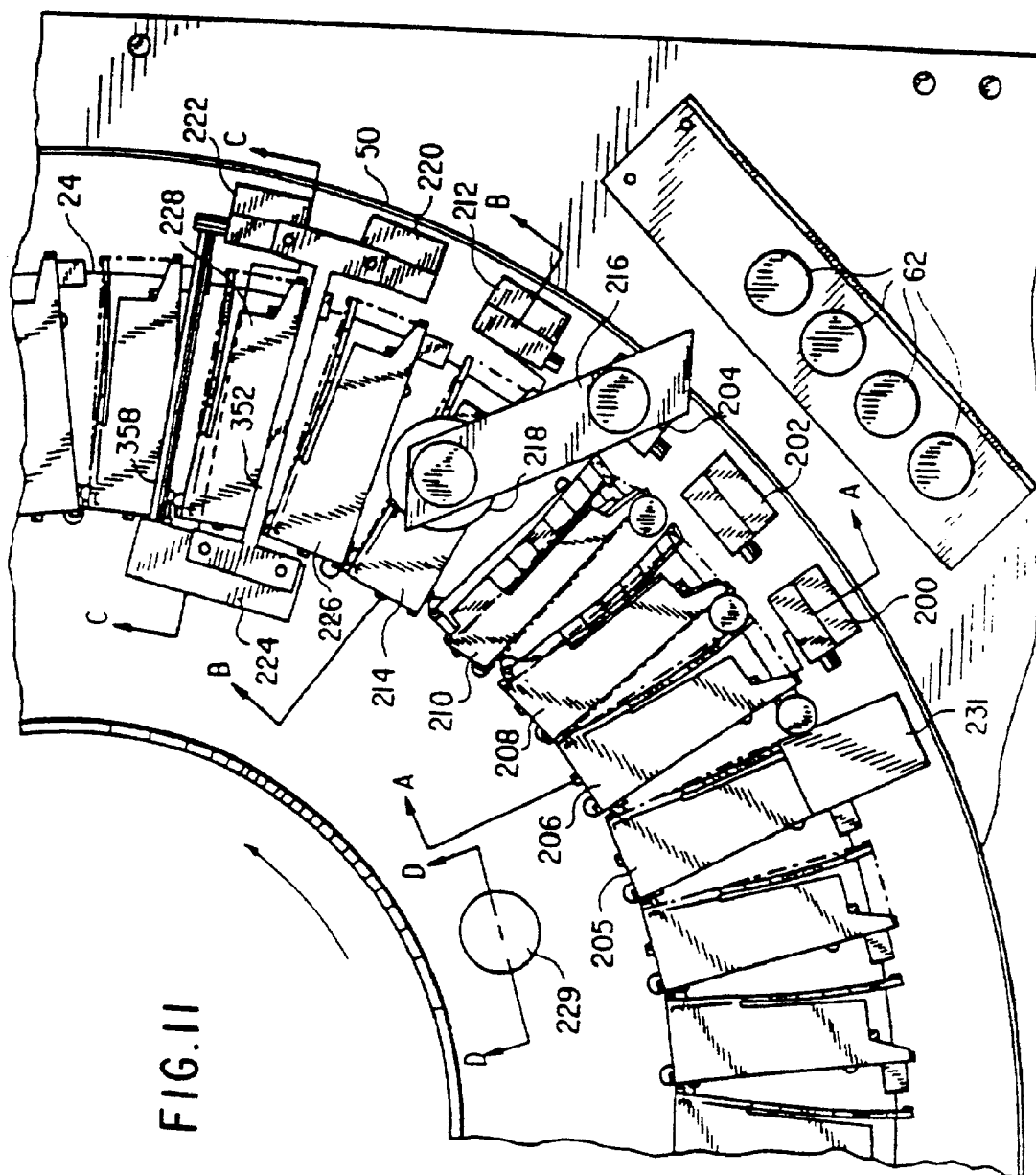


FIG. 10





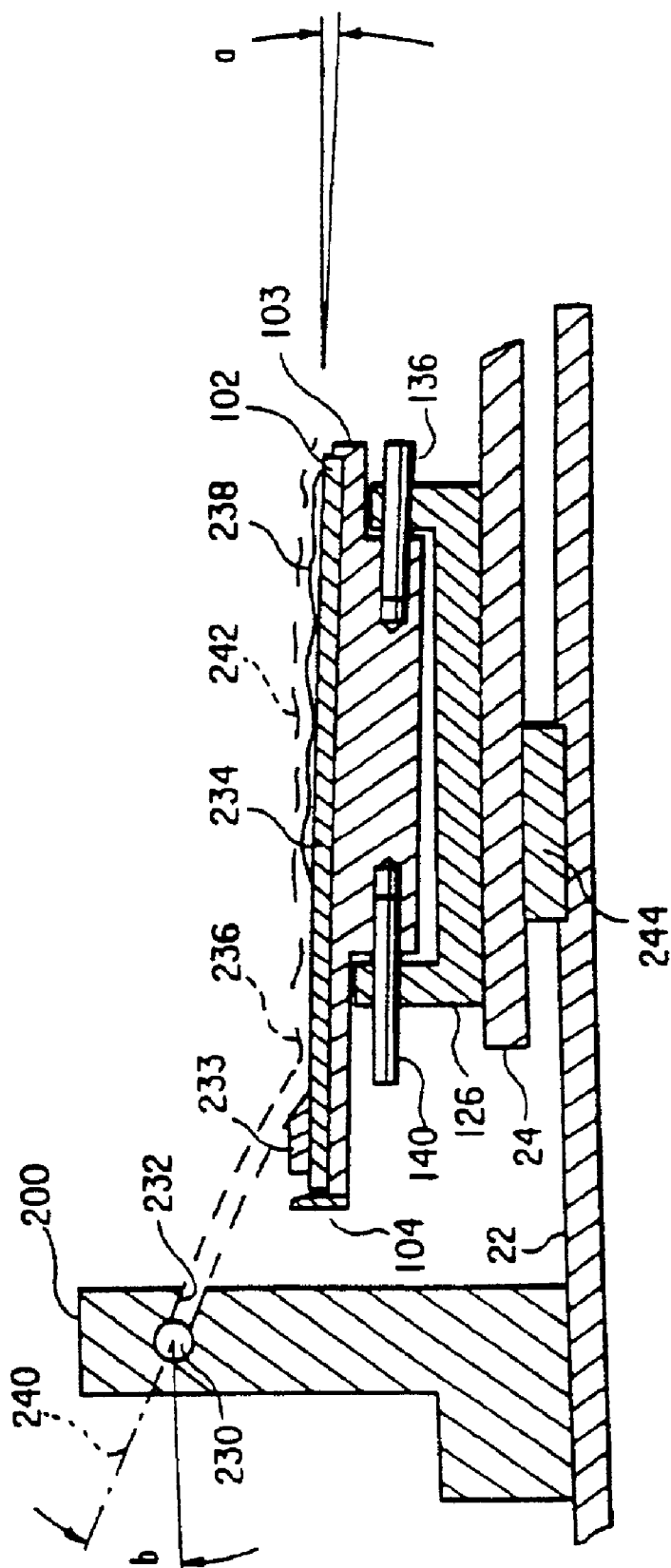


FIG. 12

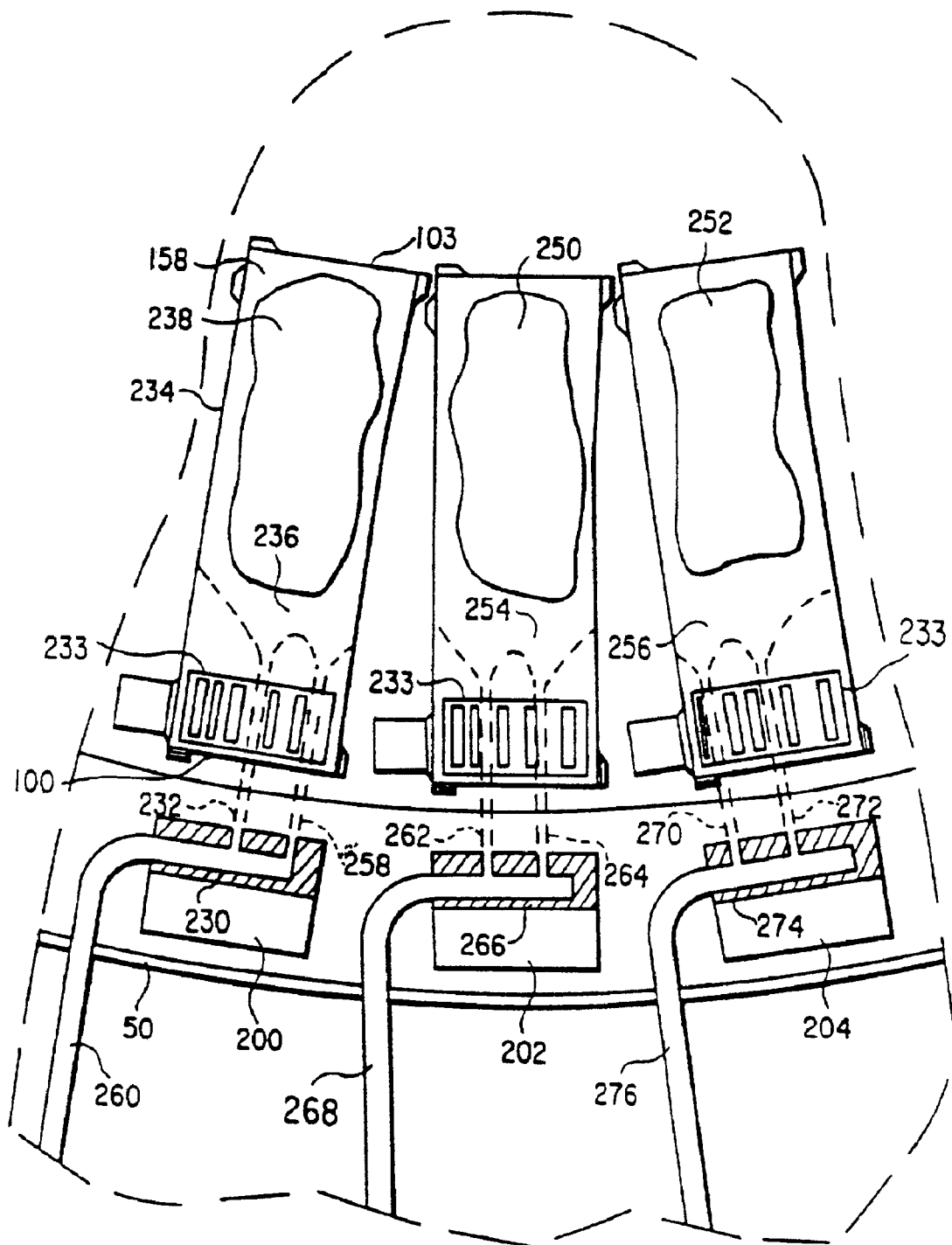
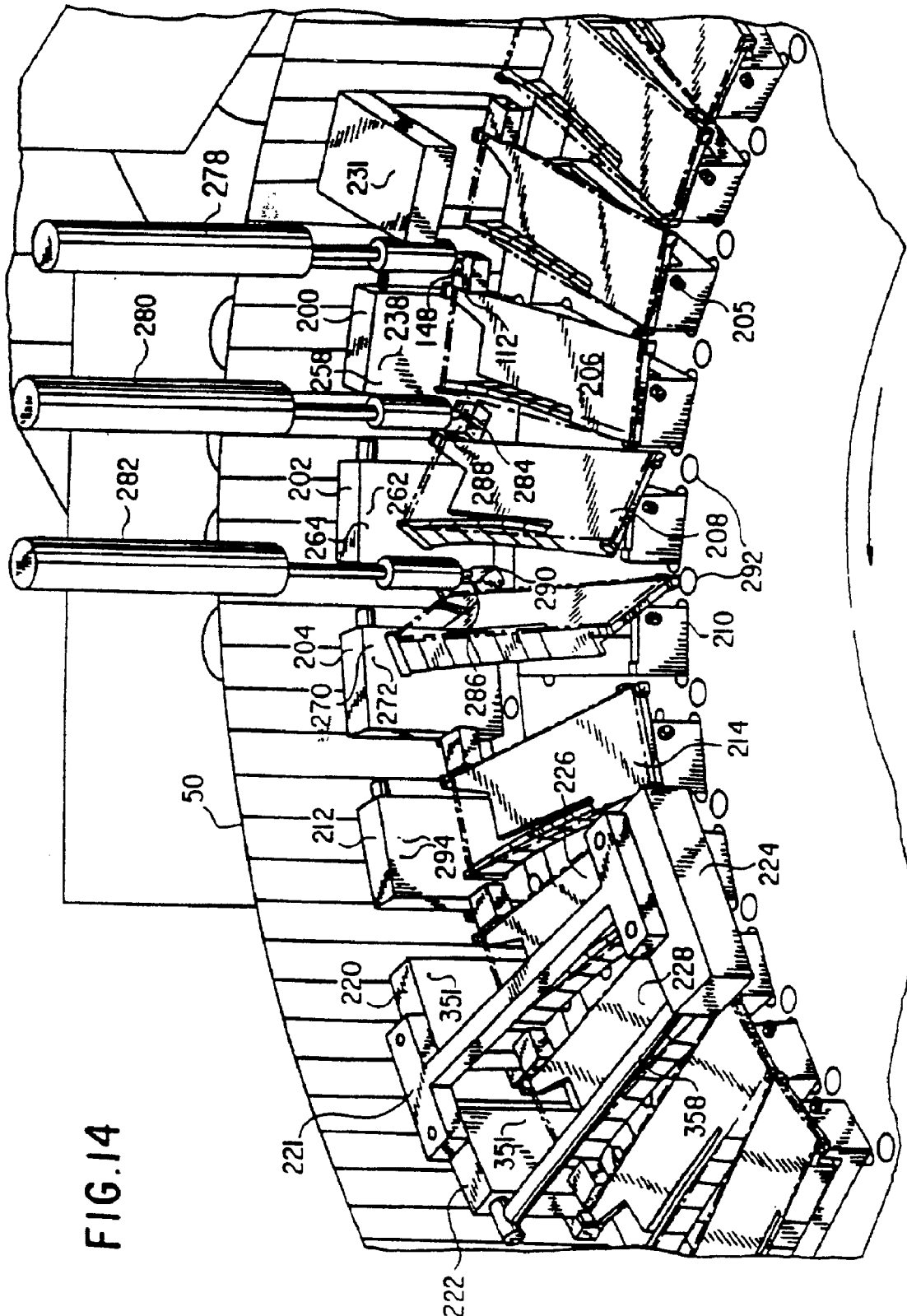


FIG. 13



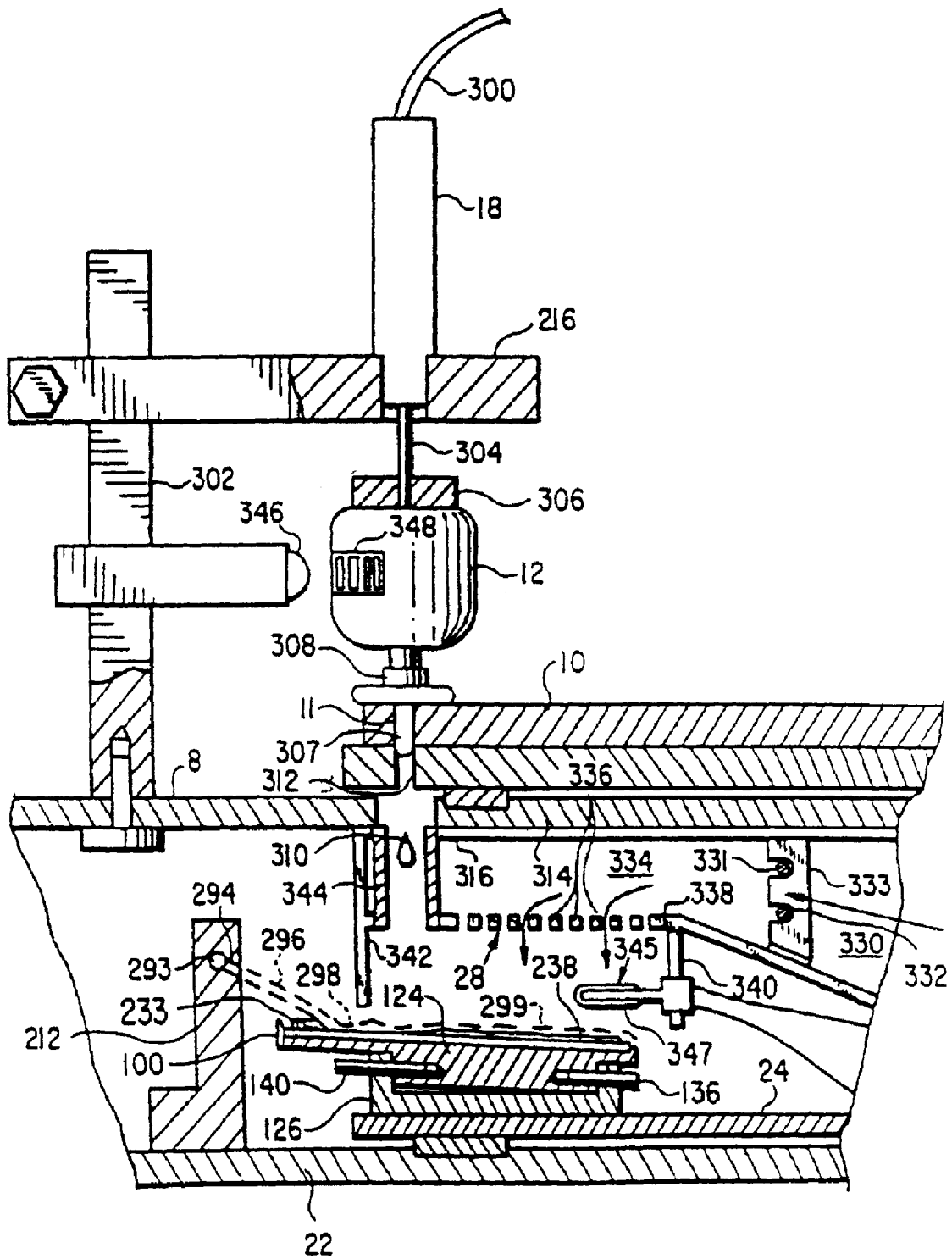
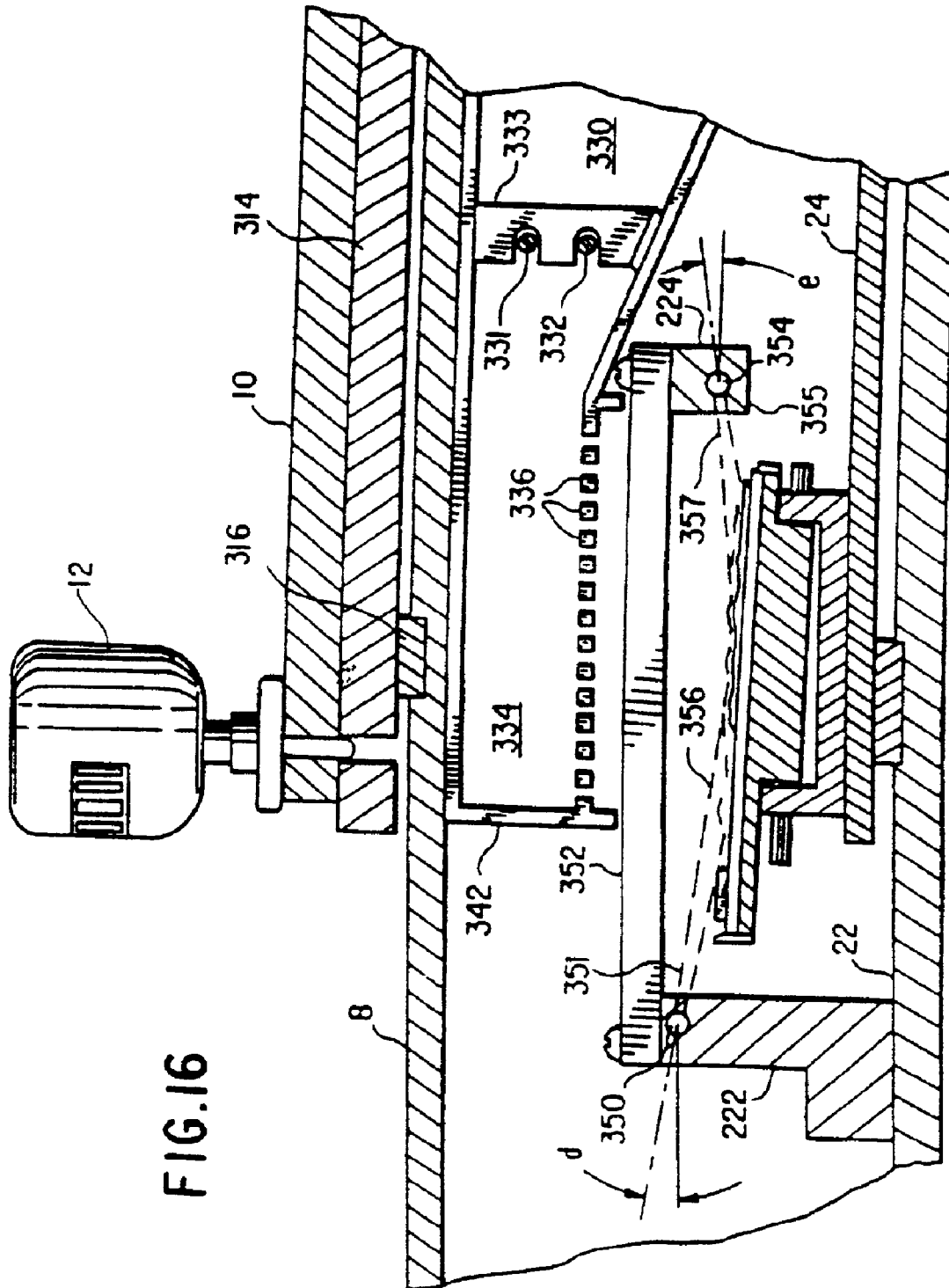


FIG. 15

FIG. 16



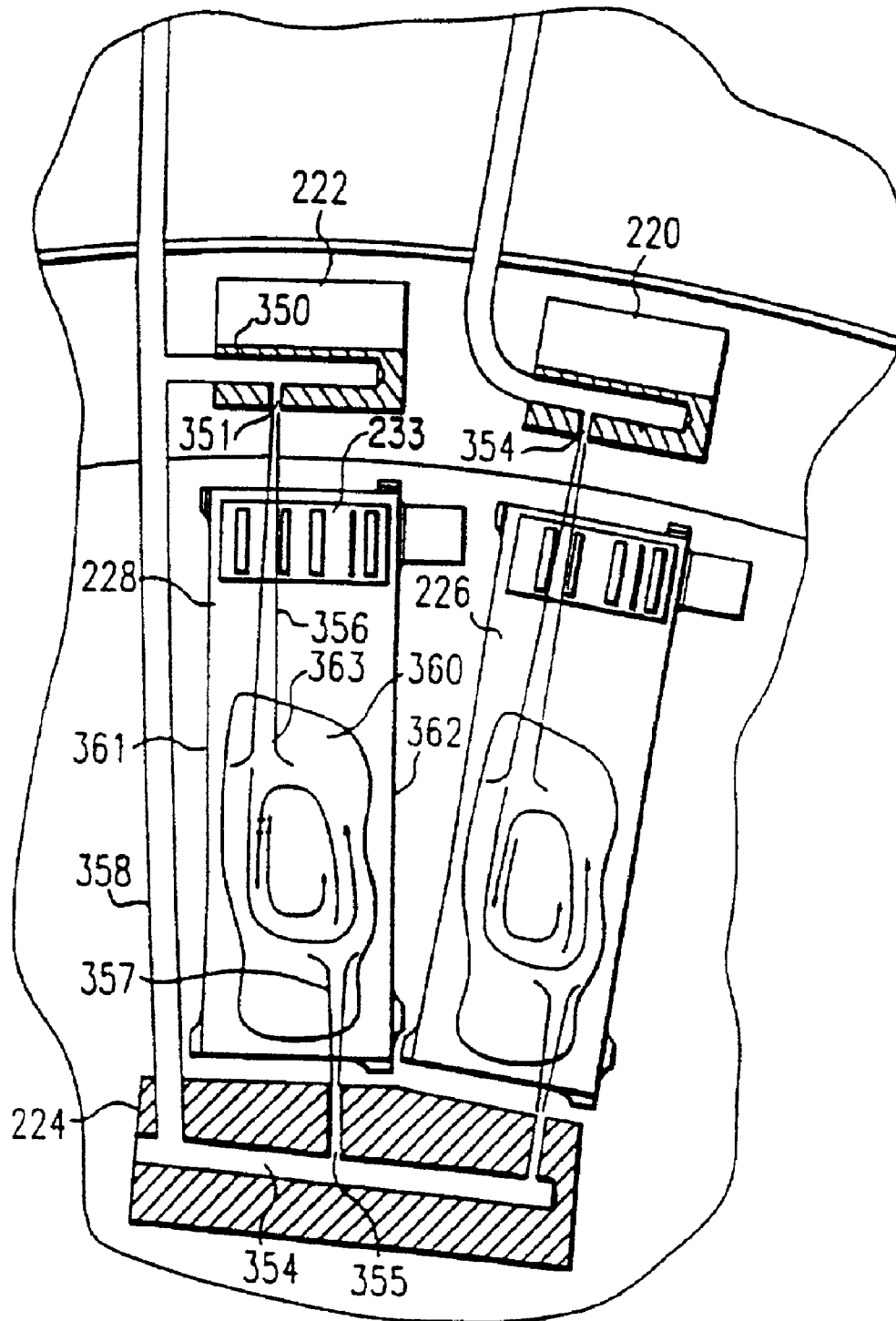


FIG. 17

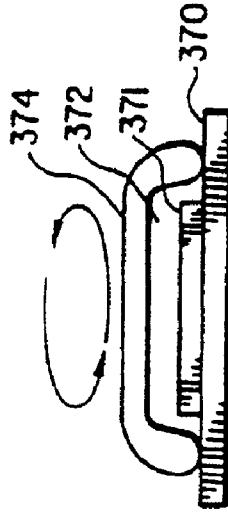


FIG. 18C

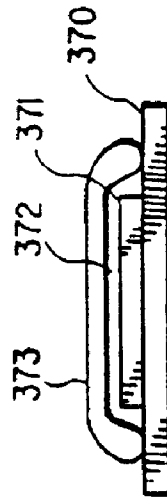


FIG. 18B

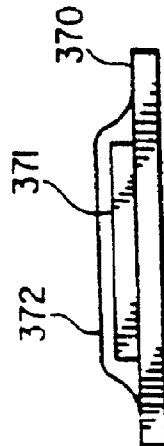


FIG. 18A

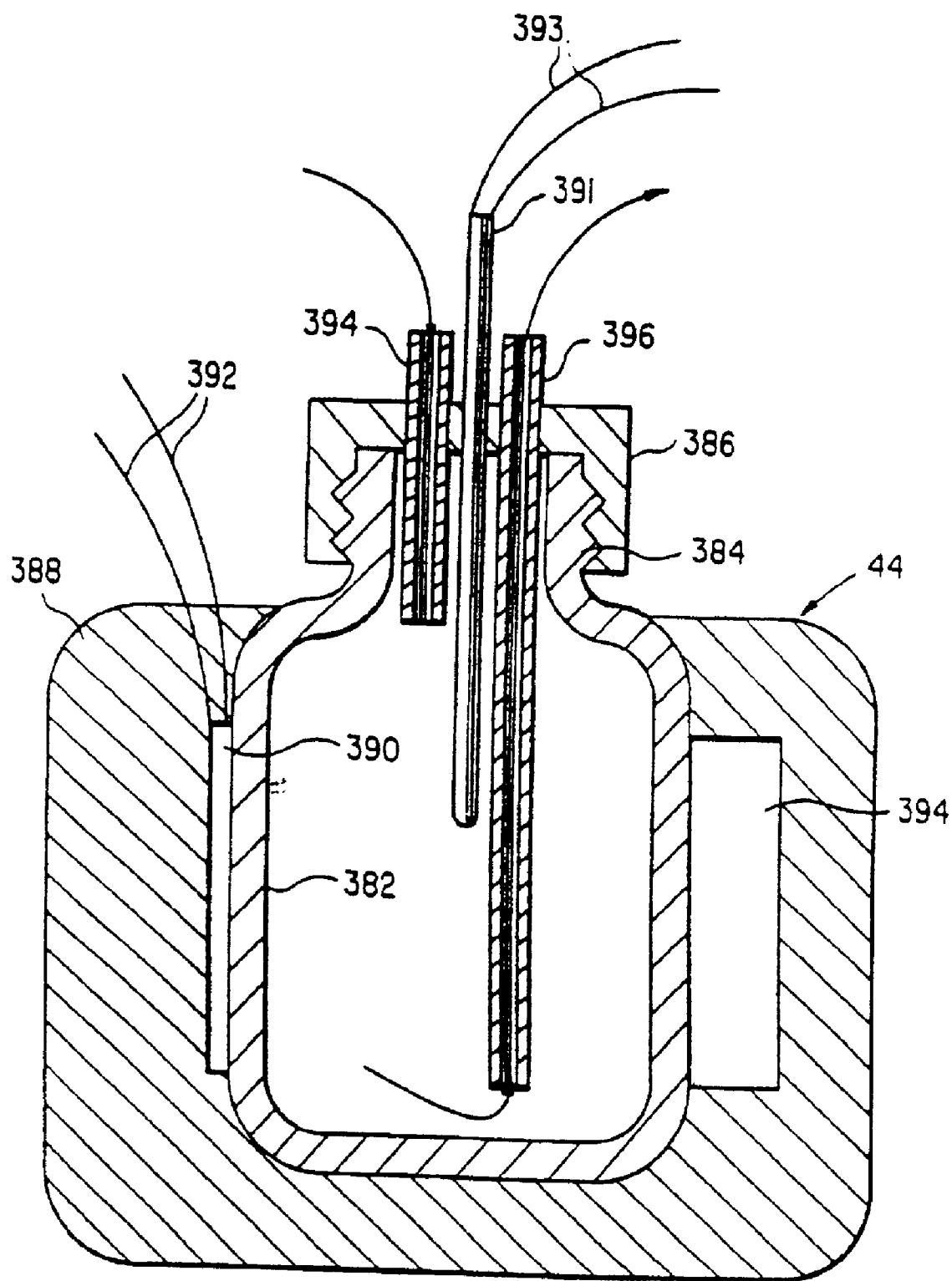


FIG. 19A

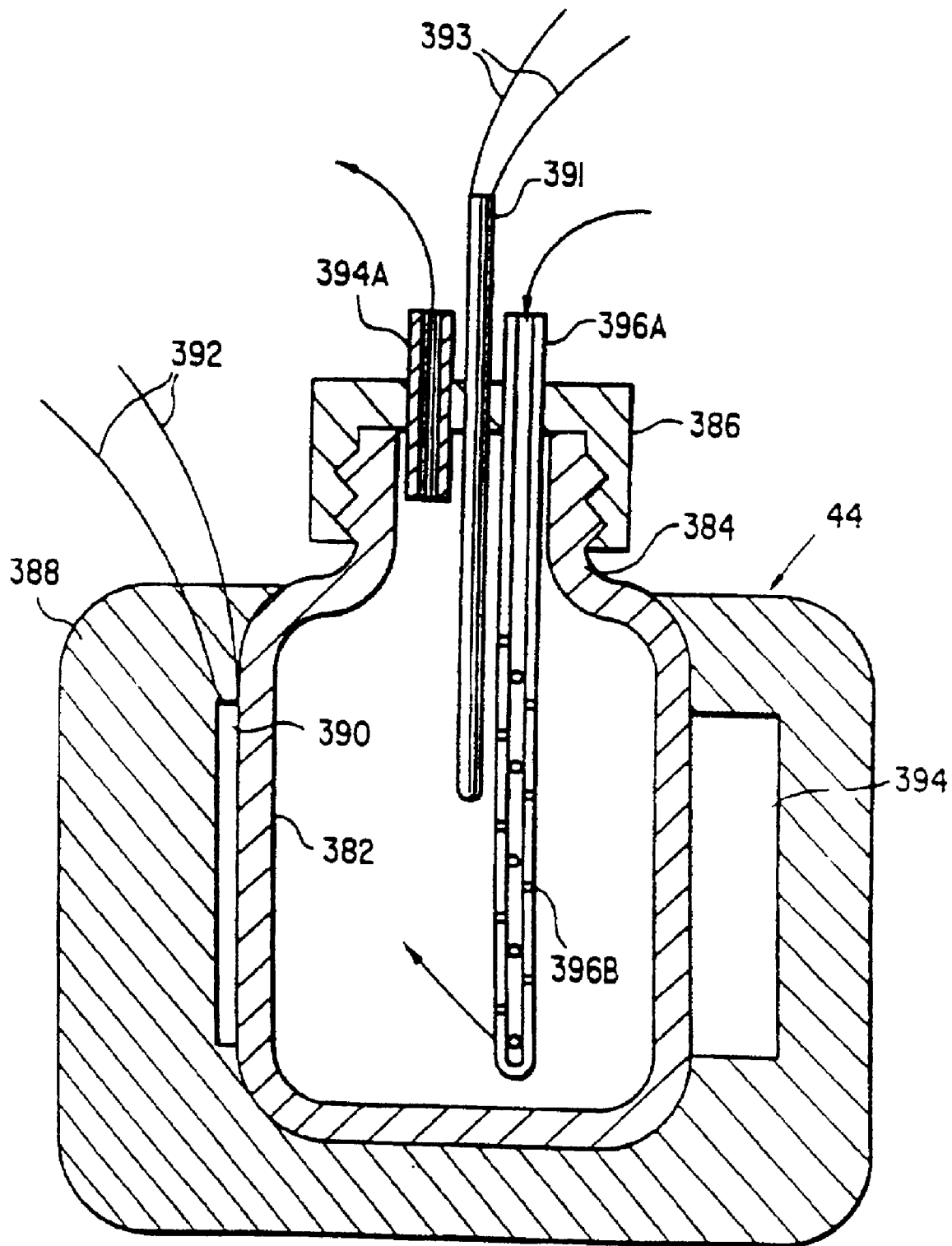


FIG. 19B

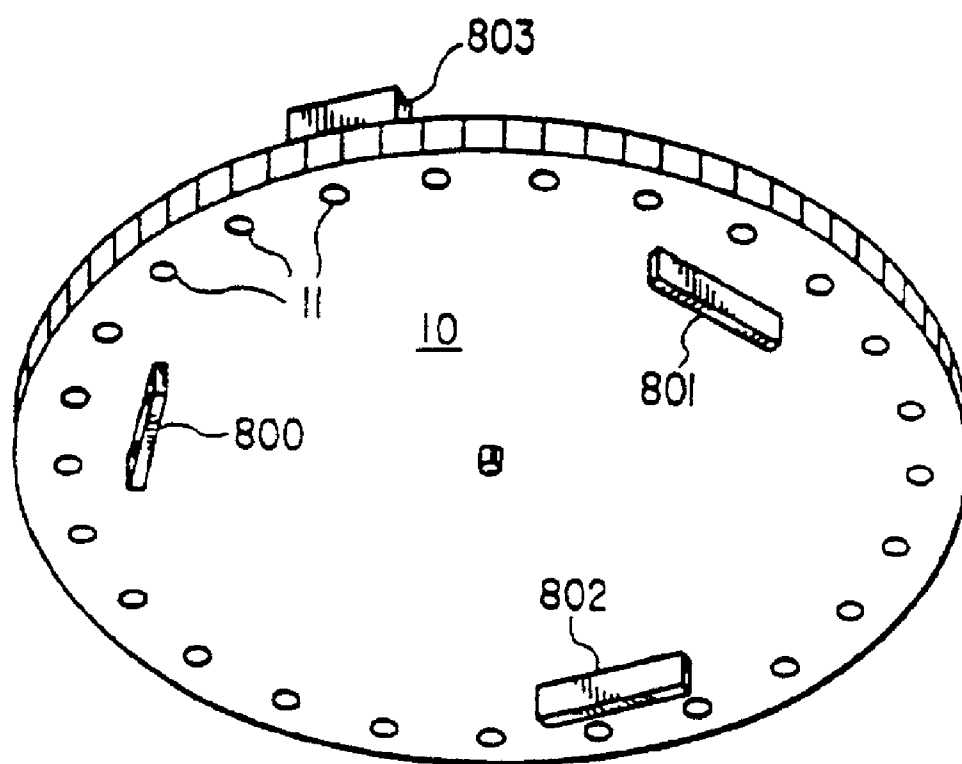


FIG. 20A

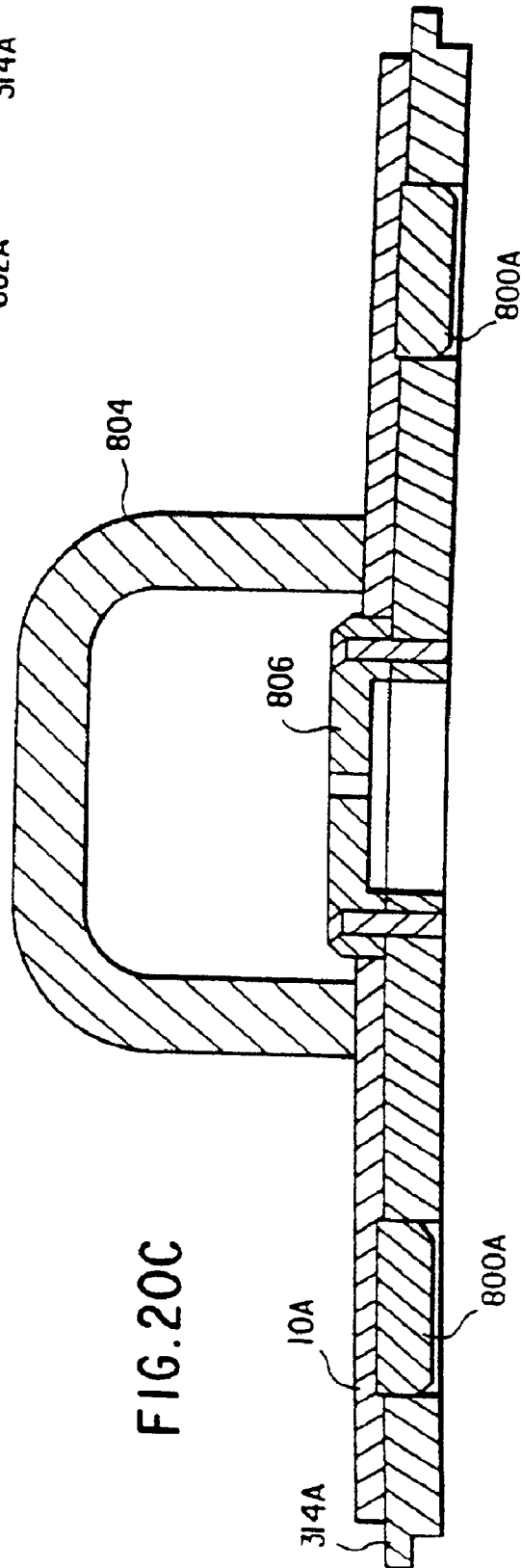
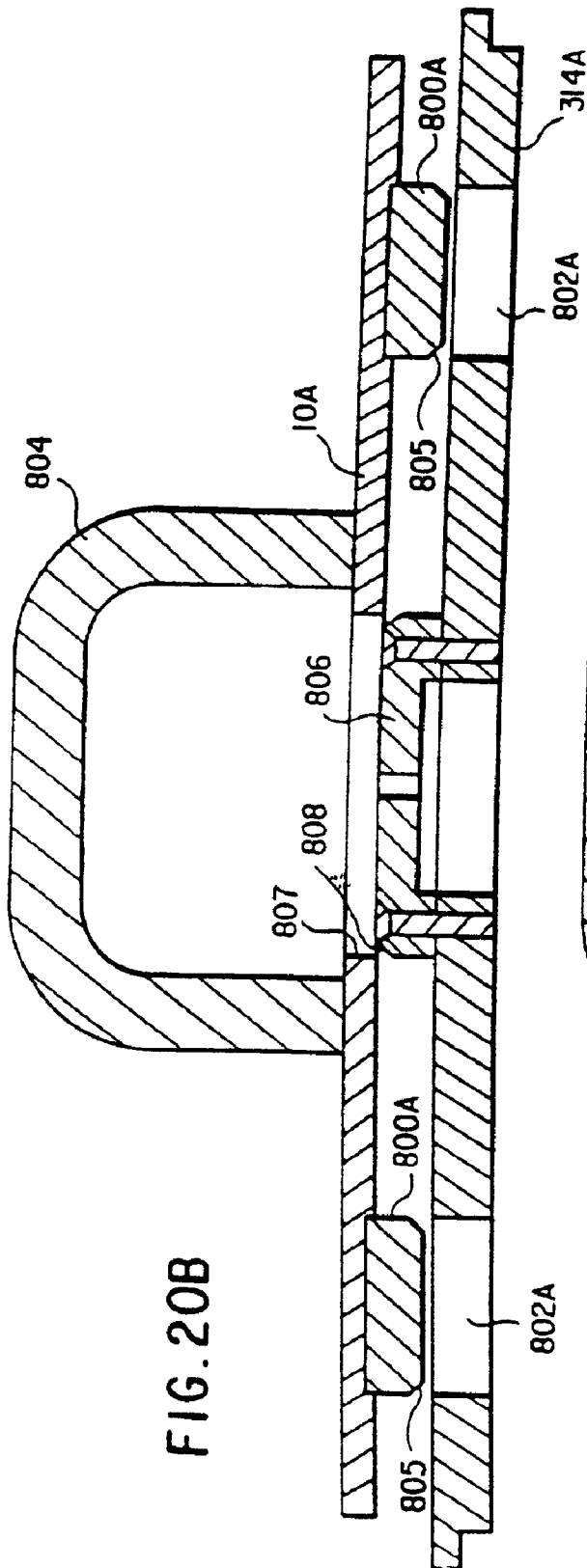
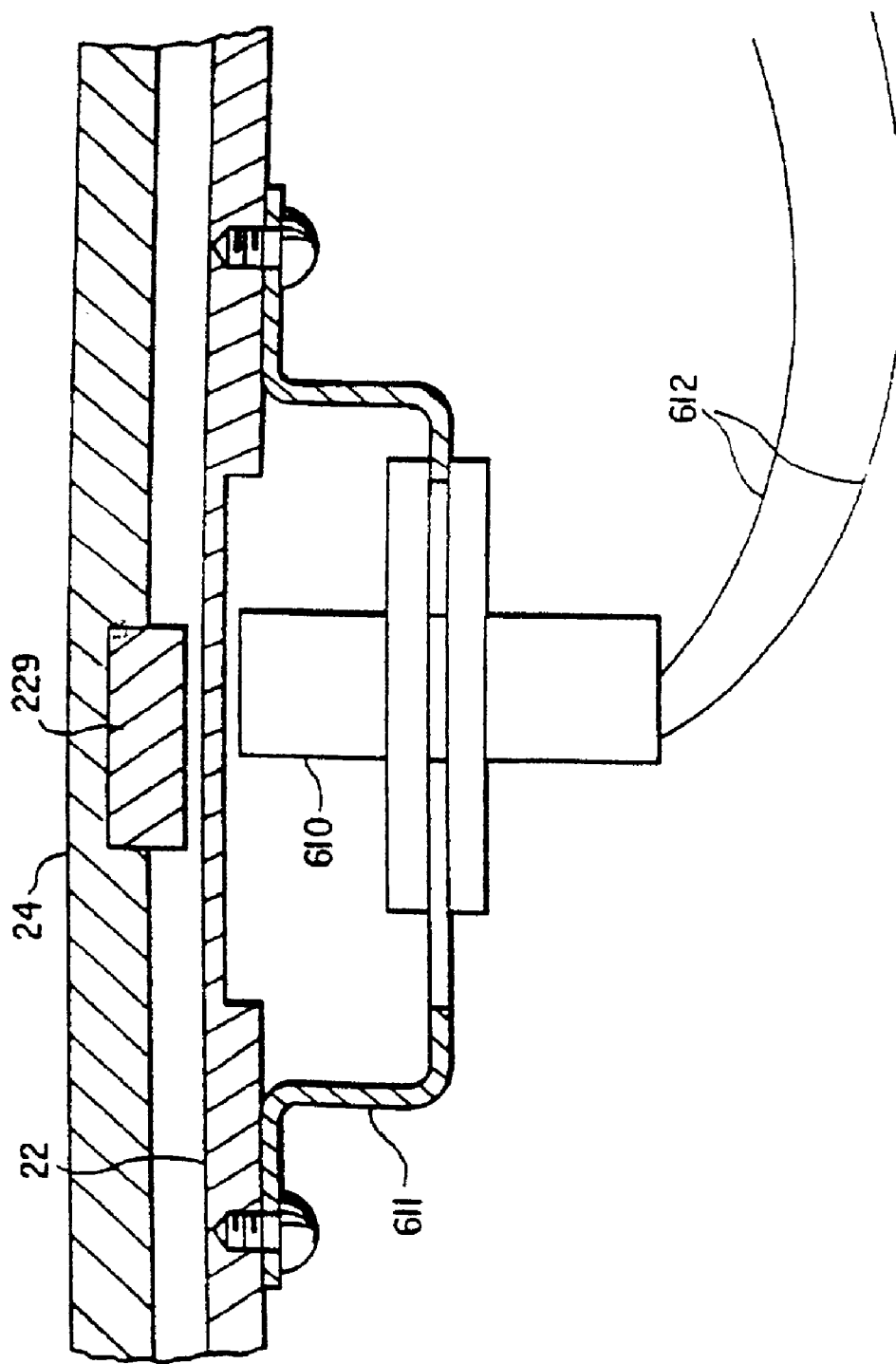
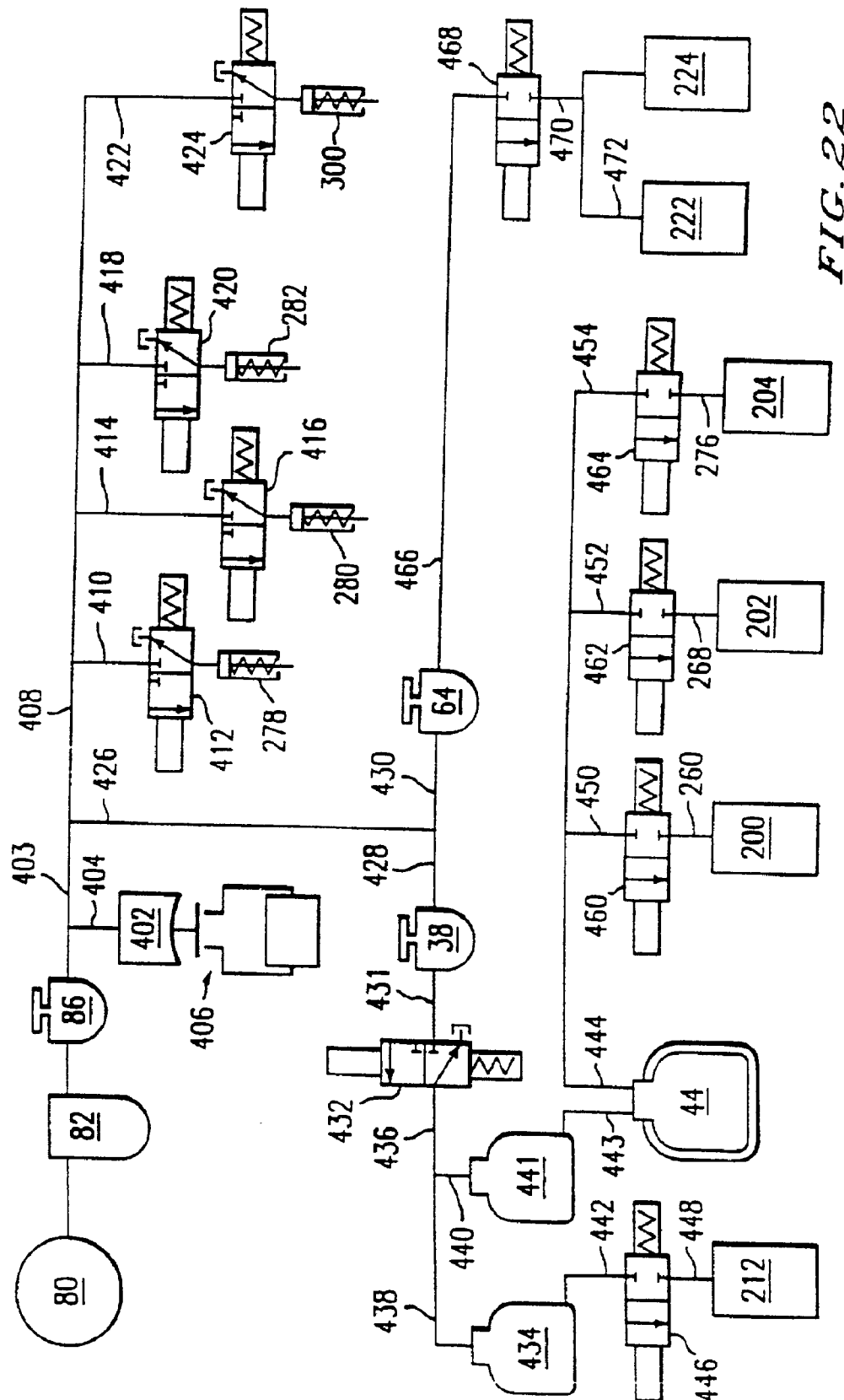
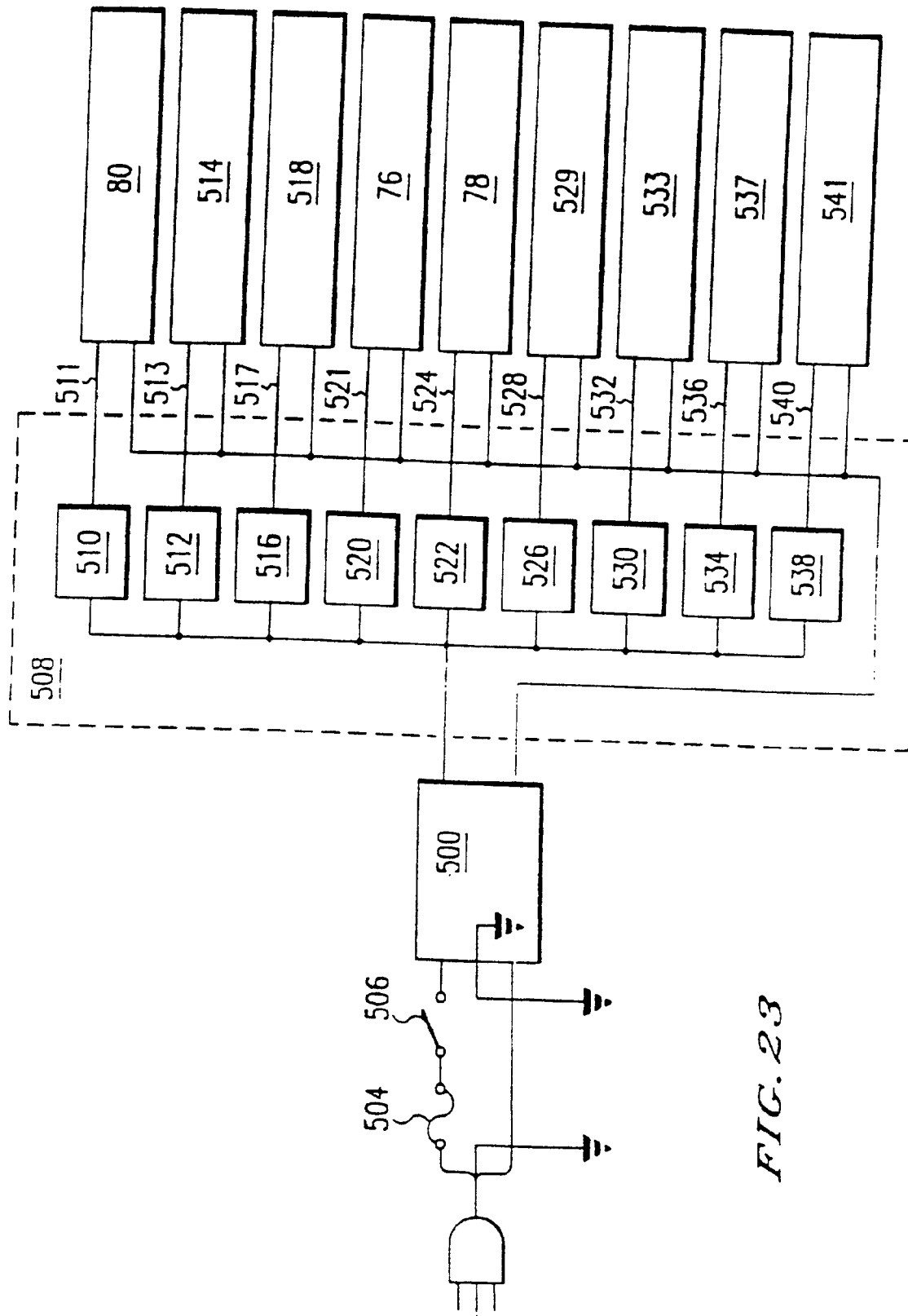


FIG. 21







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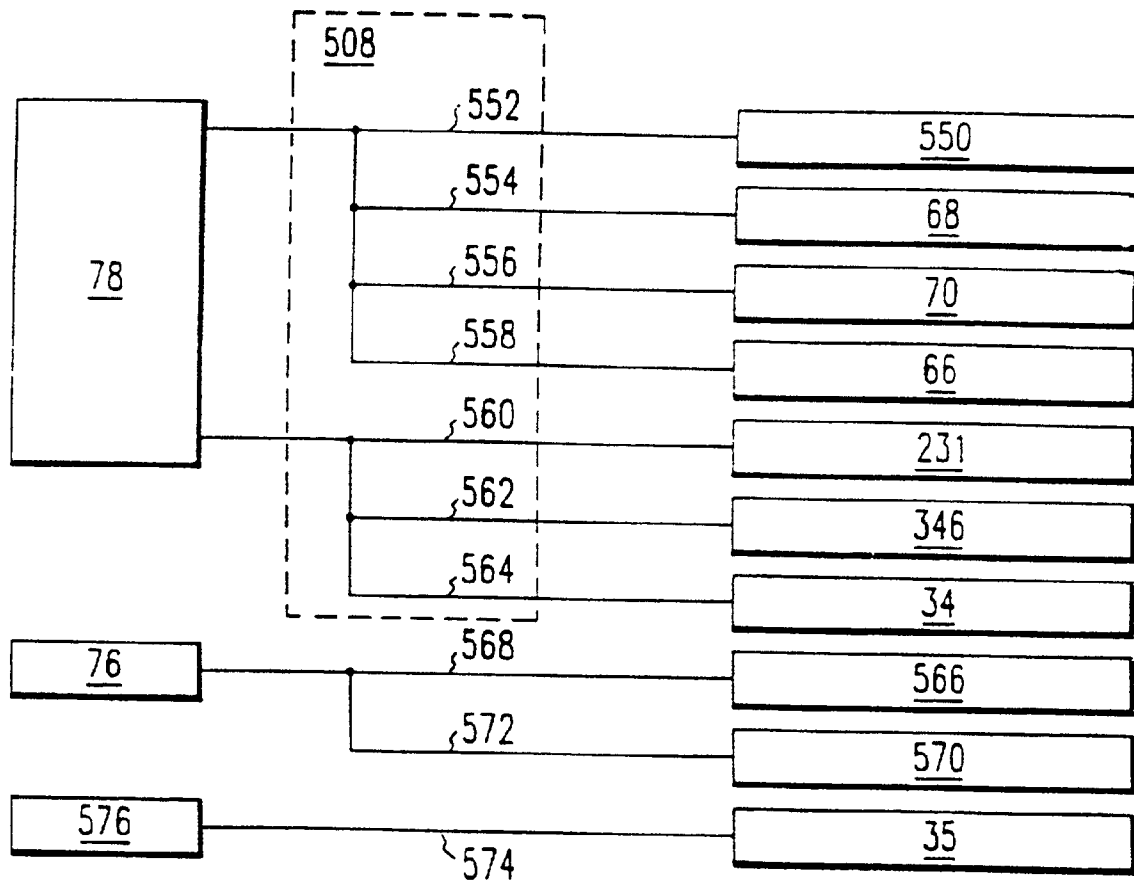
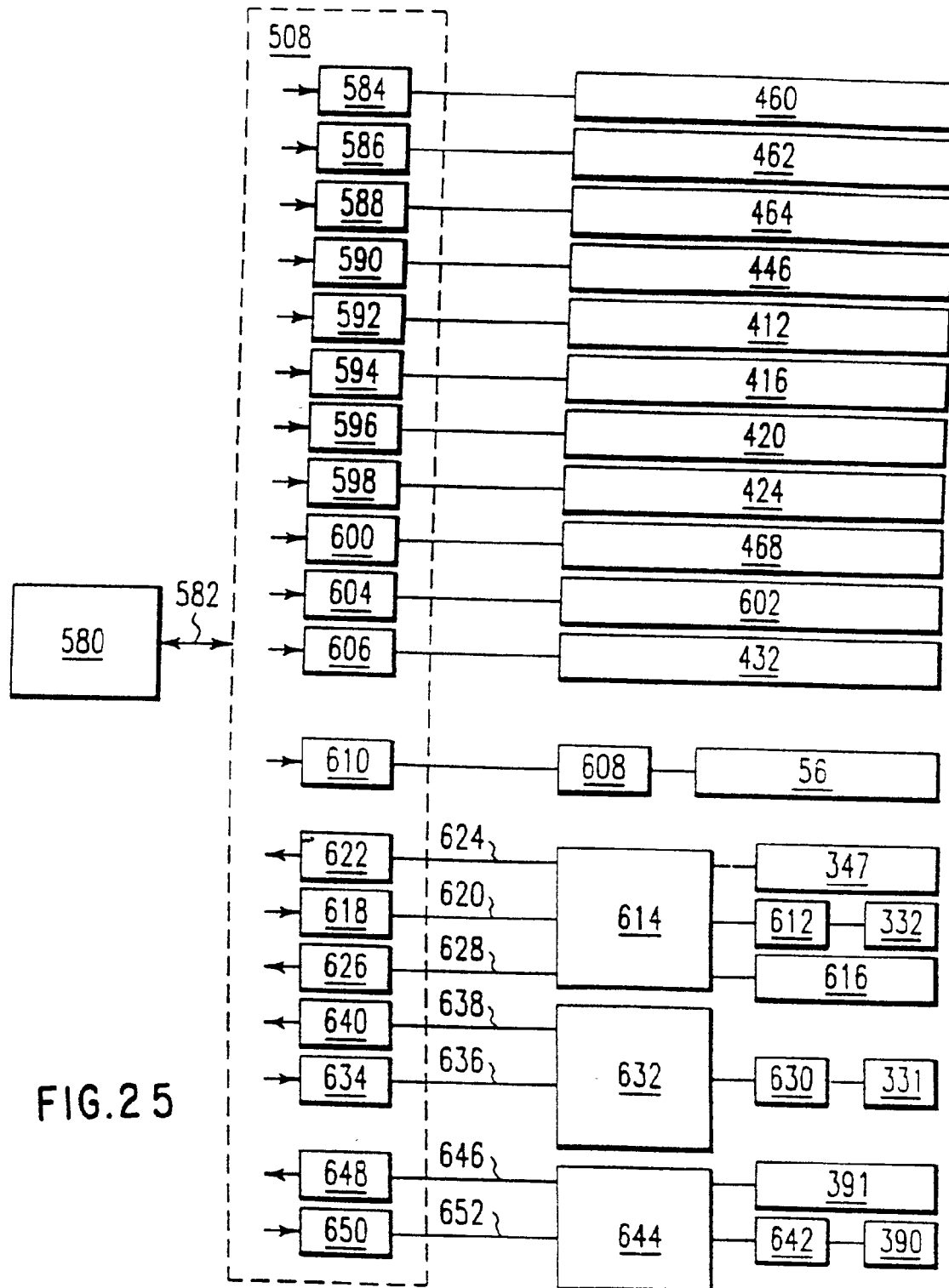


FIG. 24



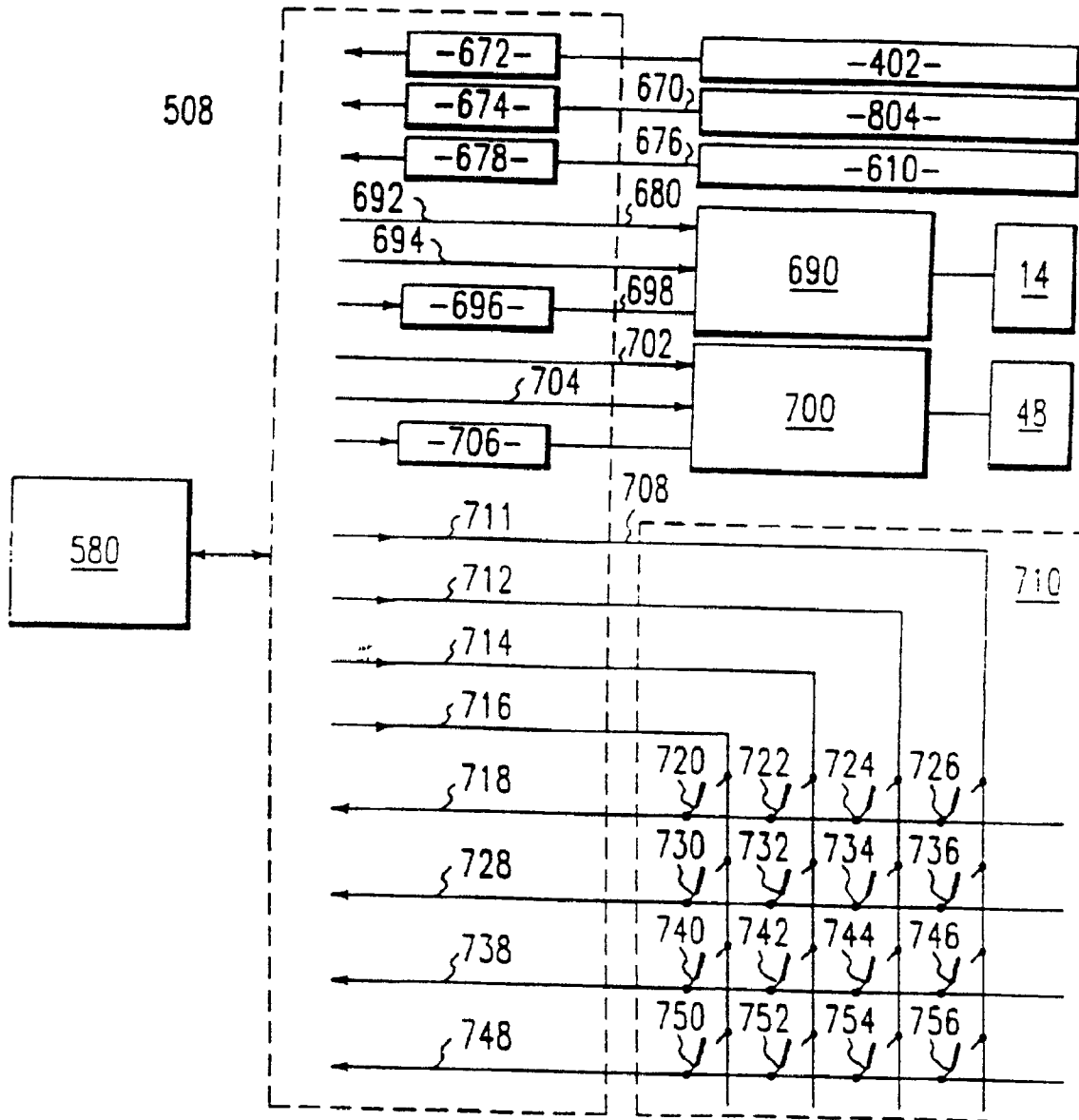


FIG. 26

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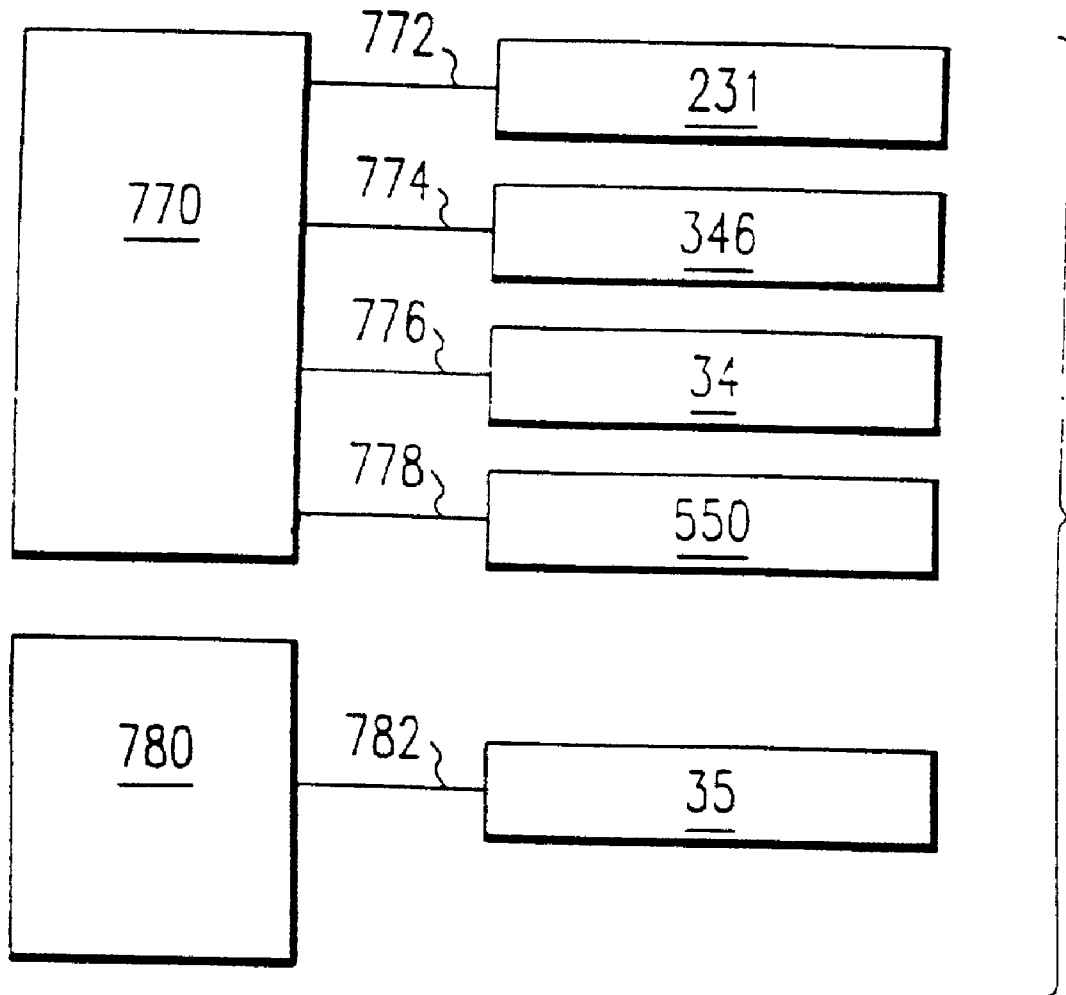


FIG. 27

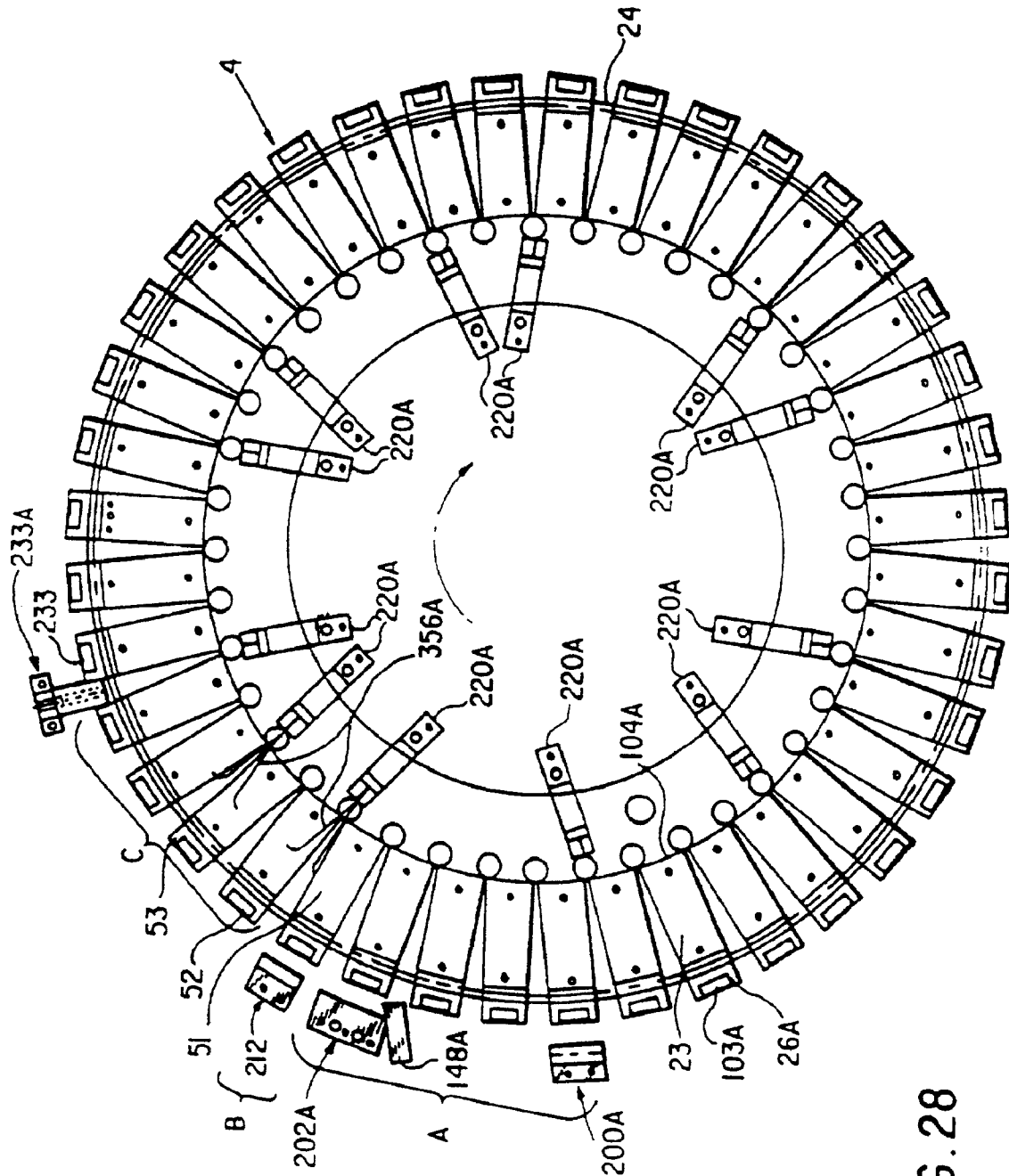


FIG. 28

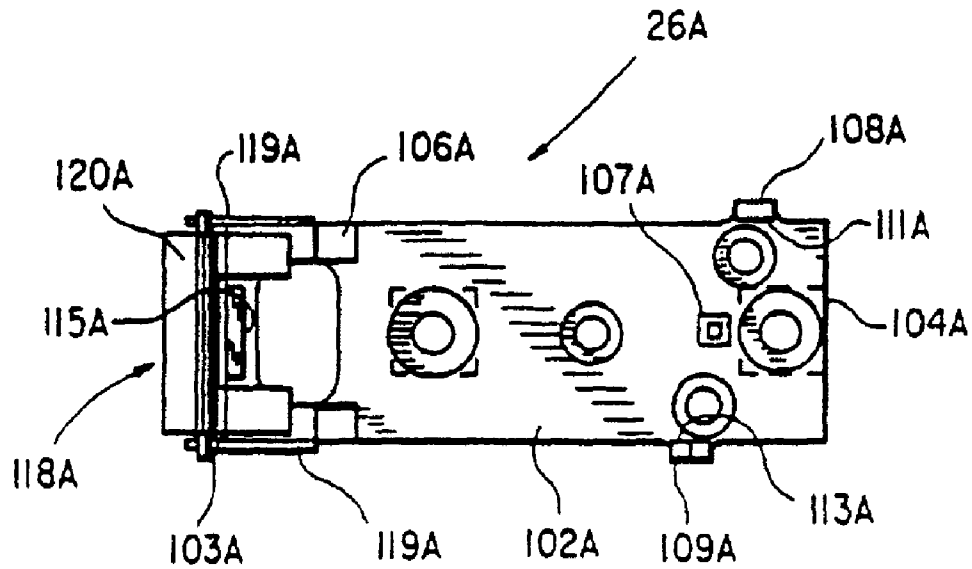


FIG. 29A

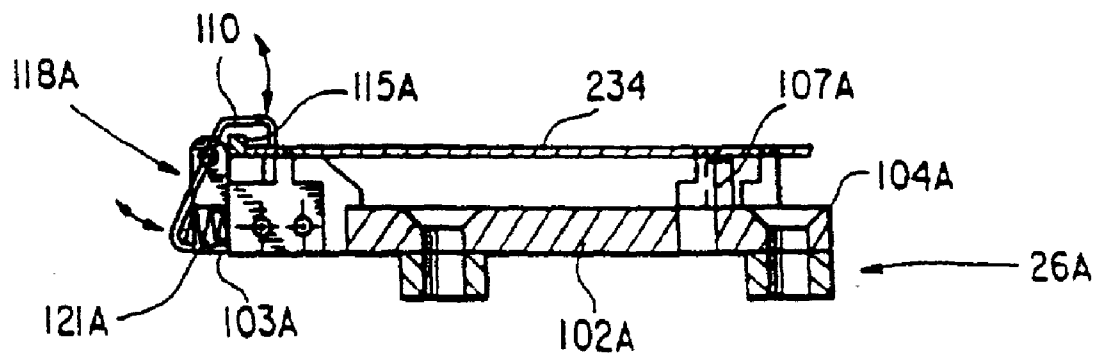
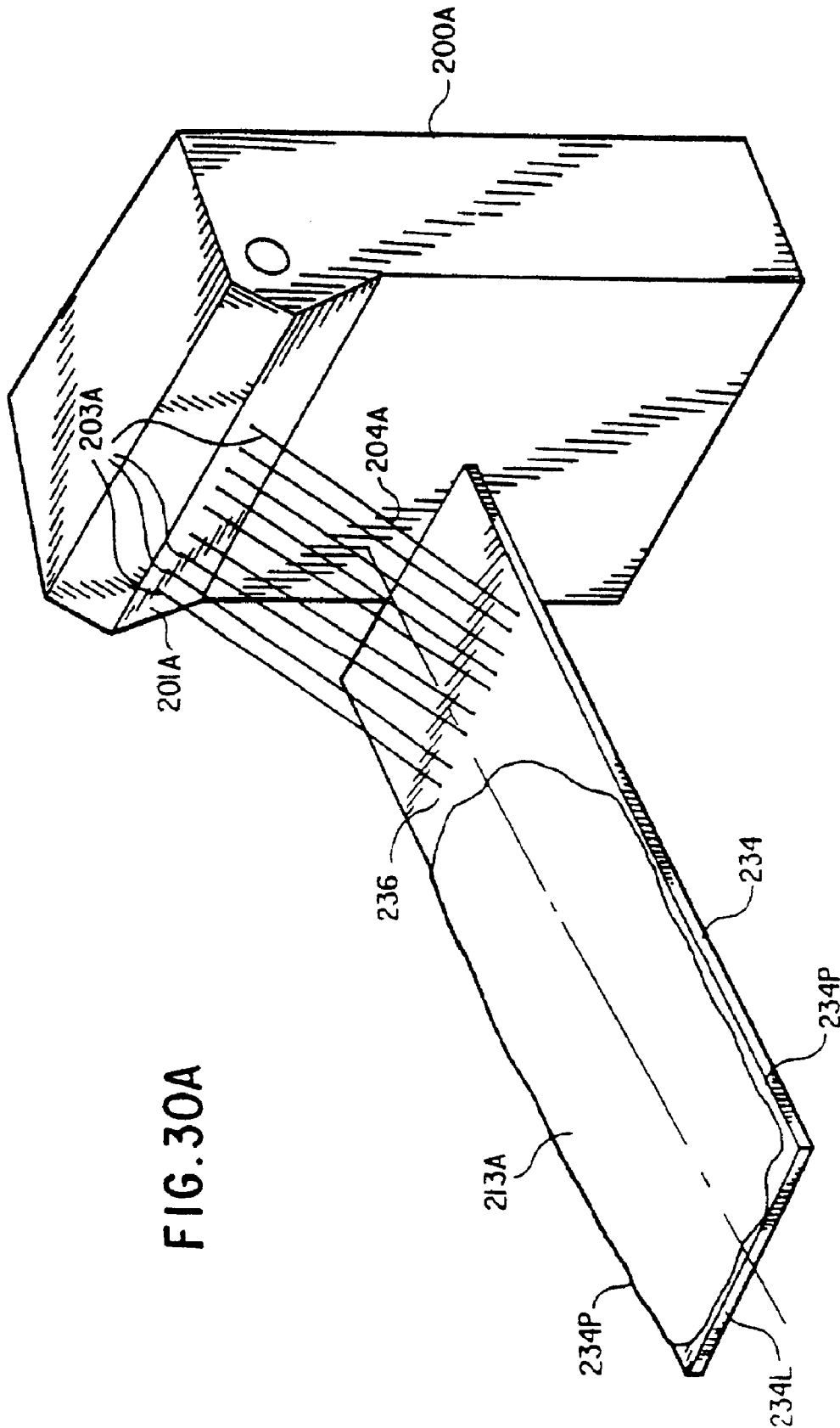


FIG. 29B



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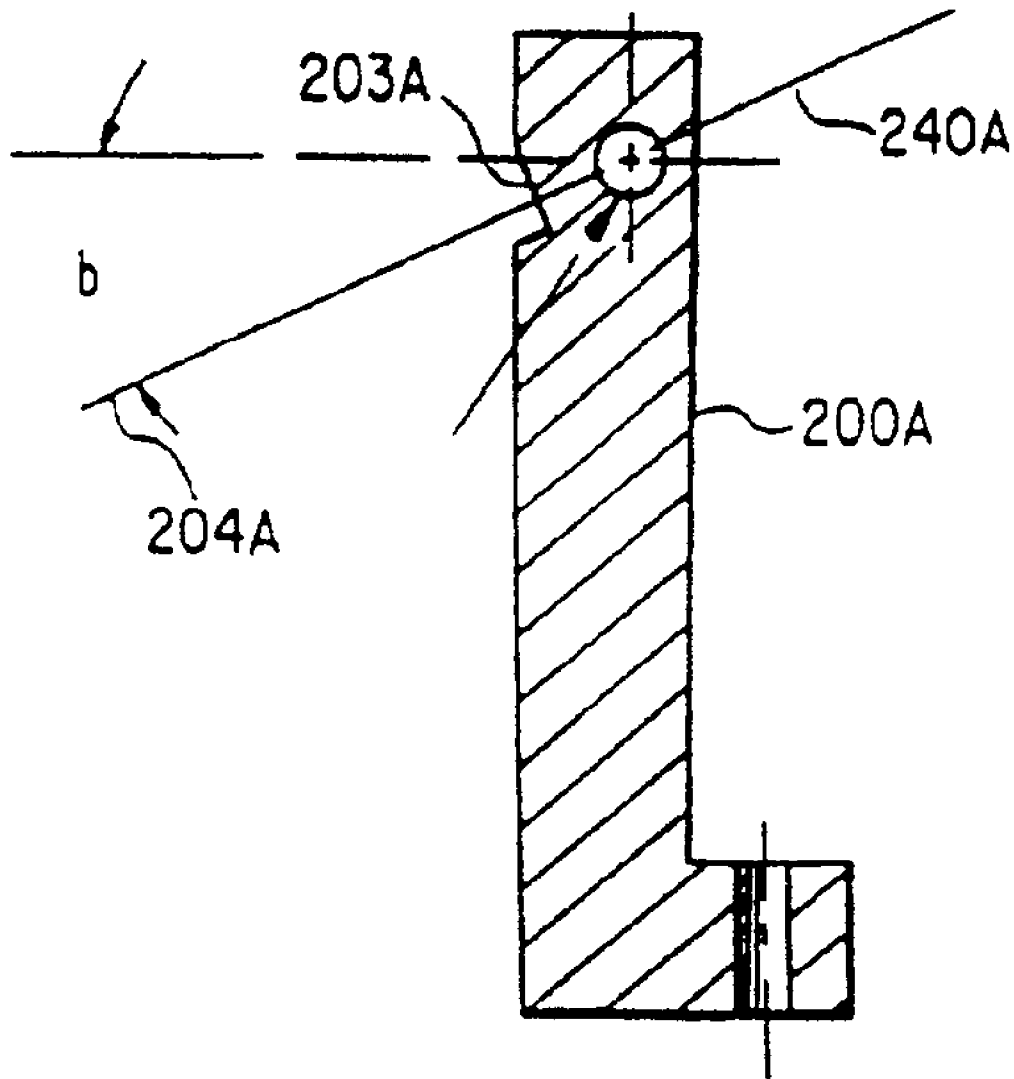


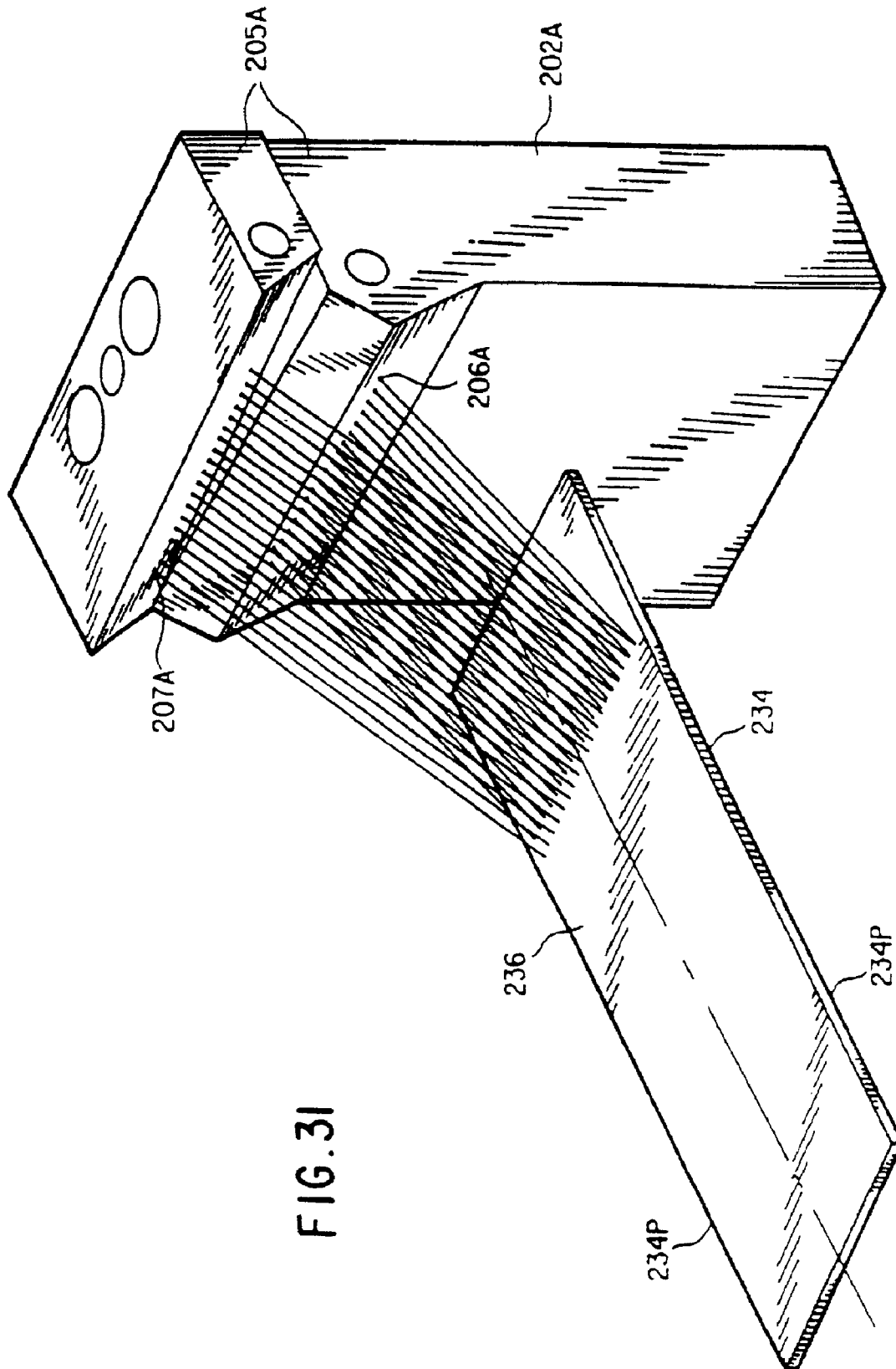
FIG. 30B

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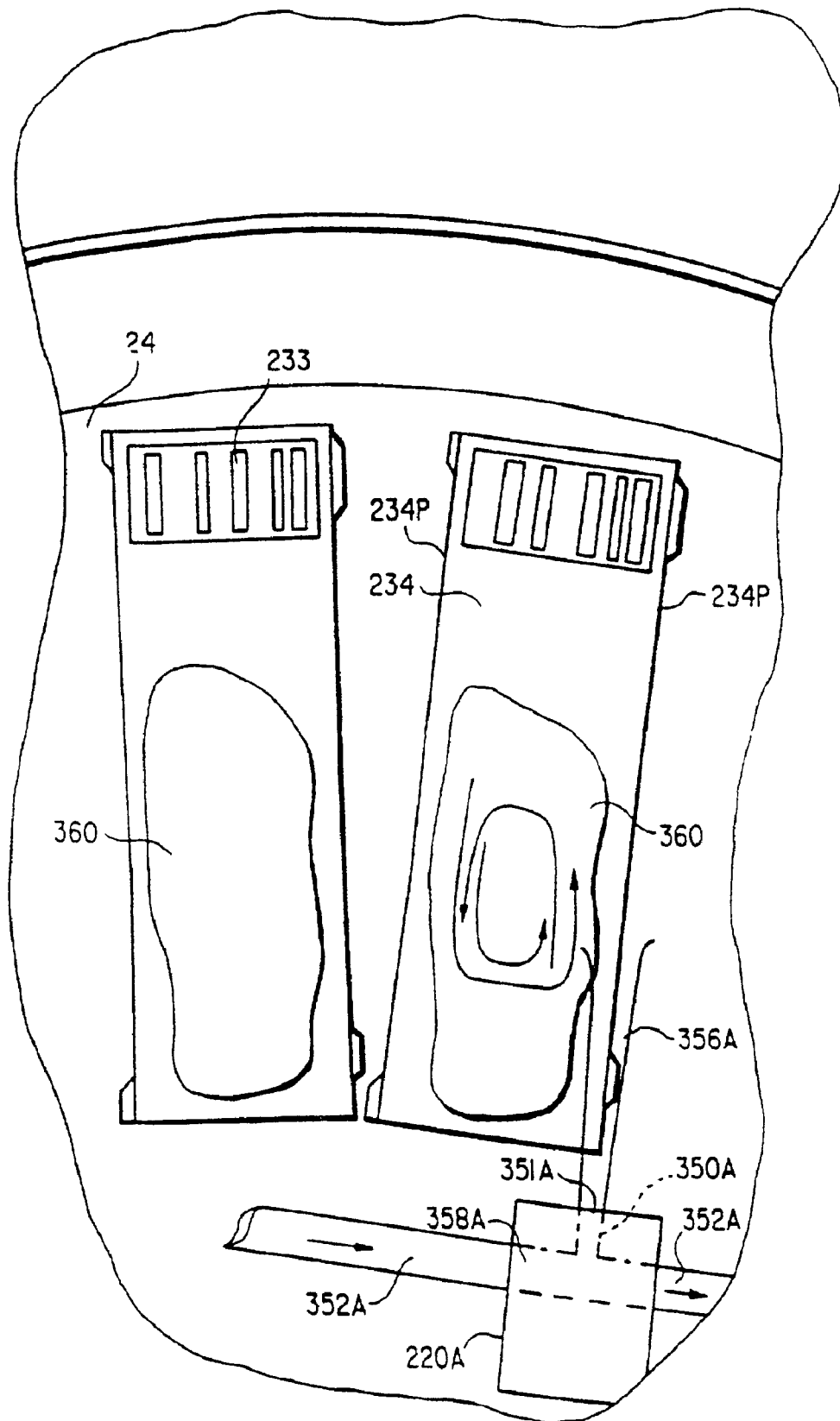


FIG. 32

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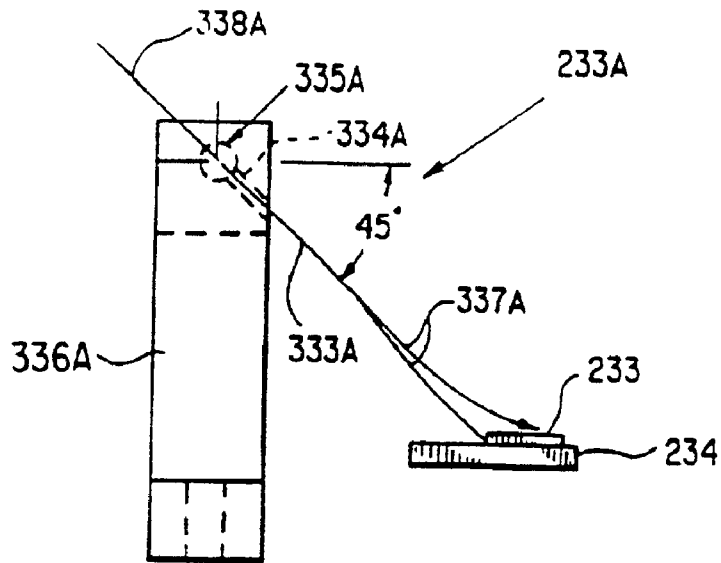


FIG. 33A

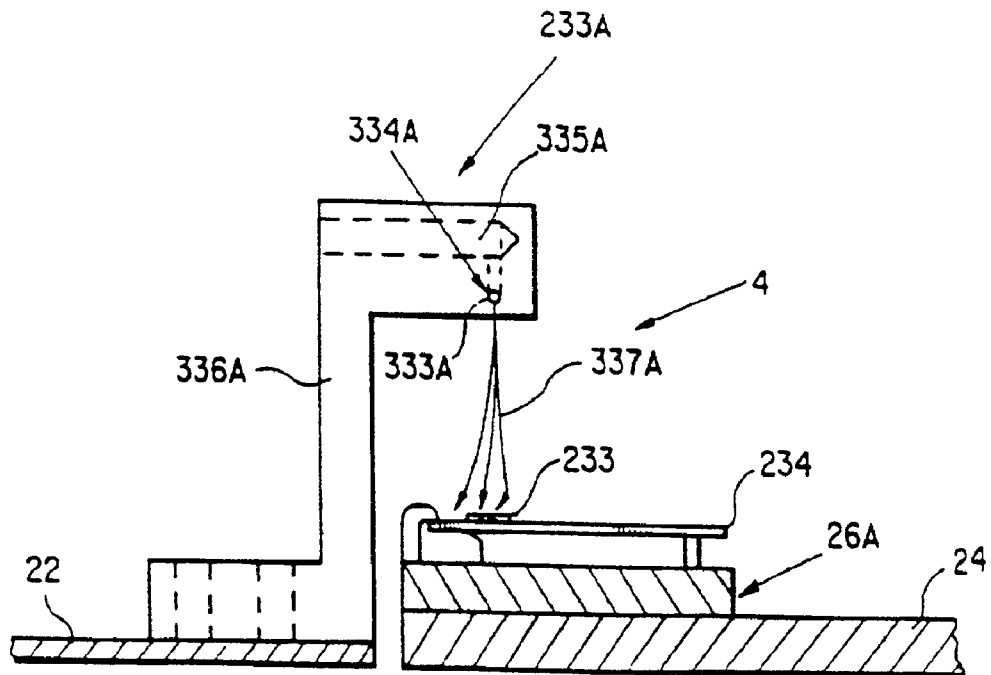


FIG. 33B

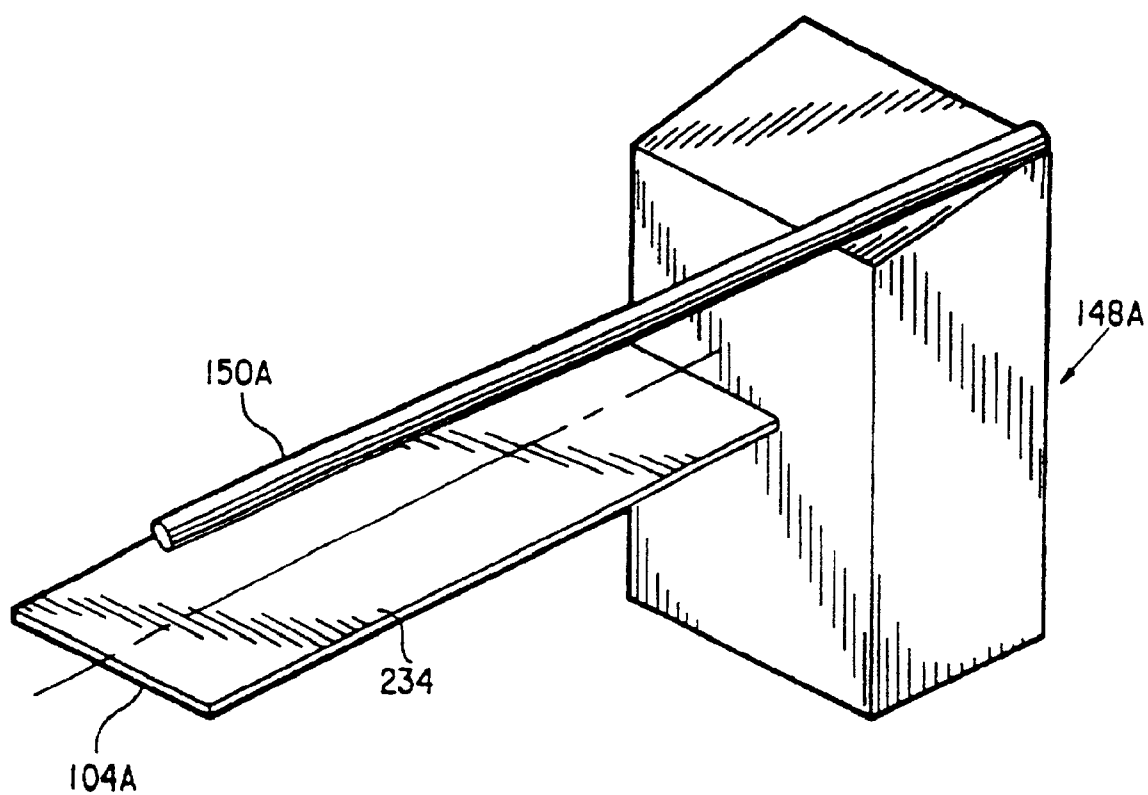


FIG. 34

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**AUTOMATED BIOLOGICAL REACTION
APPARATUS**

This is a continuation of application Ser. No. 09/452,309, filed on Dec. 1, 1999, now U.S. Pat. No. 6,352,861 which is a continuation of application Ser. No. 08/906,678, filed Aug. 5, 1997, abandoned, which is a continuation of application Ser. No. 08/479,415, filed Jun. 6, 1995, U.S. Pat. No. 5,654,200, which is a division of application Ser. No. 08/352,966, filed Dec. 9, 1994, U.S. Pat. No. 5,595,707, which is a continuation of application Ser. No. 07/924,052, filed Aug. 31, 1992, abandoned, which is a continuation-in-part of application Ser. No. 07/488,601, filed Mar. 2, 1990, abandoned.

TECHNICAL FIELD

This invention relates an improved biological reaction platform which can be used for a wide variety of assays, for example, automatic immunostaining of tissue sections, in situ DNA analysis, immunoassays such as ELISA, and the like. The automatic device of this invention can be used to process a large number of samples such as tissue sections mounted on slide surfaces using agents and protocols pre-selected by the operator, while maintaining the slide surfaces in a substantially horizontal plane throughout the incubation cycles.

BACKGROUND ART

Immunostaining and in situ DNA analysis are useful tools in histological diagnosis and the study of tissue morphology. Immunostaining relies on the specific binding affinity of antibodies with epitopes in tissue samples, and the increasing availability of antibodies which bind specifically with unique epitopes present only in certain types of diseased cellular tissue. Immunostaining requires a series of treatment steps conducted on a tissue section mounted on a glass slide to highlight by selective staining certain morphological indicators of disease states. Typical steps include pretreatment of the tissue section to reduce non-specific binding, antibody treatment and incubation, enzyme labeled secondary antibody treatment and incubation, substrate reaction with the enzyme to produce a fluorophore or chromophore highlighting areas of the tissue section having epitopes binding with the antibody, counterstaining, and the like. Each of these steps is separated by multiple rinse steps to remove unreacted residual reagent from the prior step. Incubations are conducted at elevated temperatures, usually around 40° C., and the tissue must be continuously protected from dehydration. In situ DNA analysis relies upon the specific binding affinity of probes with unique nucleotide sequences in cell or tissue samples and similarly involves a series of process steps, with a variety of reagents and process temperature requirements.

Automated systems have been explored to introduce cost savings, uniformity of slide preparation, and reduction of procedural human errors. Stross, W. et al, *J.Clin.Pathol.* 42:106-112 (1989) describes a system comprising a series of baths positioned under the circumference of a circular, rotatable disc from which slide trays are suspended. The disc is lifted to lift slide trays from their baths, turned to position the slide trays above the next consecutive bath, and lowered to immerse the slide trays in the baths. This operation can be automated with suitable timers and switches. This system exposes each of the slides to the same treatment and relies on dipping for application of reactants and rinsing.

Stark, E. et al, *J.Immunol.Methods.* 107:89-92 (1988) describes a microprocessor controlled system including a

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revolving table or carousel supporting radially positioned slides. A stepper motor rotates the table, placing each slide under one of the stationary syringes positioned above the slides. A predetermined volume of liquid, determined by a dial, is delivered to a slide from each syringe. Microprocessor controls are provided.

Cosgrove, R. et al, *ACL*, pp 23-27 (December, 1989) describe an immunostaining apparatus for auto-pipetting reagents into a slide well from a carousel holding up to 18 reagent vials. Below each well, a coverplate spaced from the surface of each slide provides cover and defines a reagent flow channel. The slides are suspended at a steep angle. Reagent from the well flows downward over the slide surface. A row of slides are suspended for sequential treatment. Washing is accomplished by a 3 to 4 minute continuous running wash over the sample, yielding an estimated 20:1 wash/reagent ratio.

Brigati, D. et al, *J.Histotechnology* 11:165-183 (1988) and Unger, E., Brigati, D. et al, *J.Histotechnology*. 11:253-258 (1988) describe the Fisher automated work station using capillary gap technology. A coverplate is placed over the slide, forming a capillary gap. Liquid is introduced into the capillary gap by placing the lower edge of the plate-slide pair in a liquid. Liquid is removed by placing the lower edge of the plate-slide pair on a blotter. The system is further described in U.S. Pat. Nos. 4,777,020, 4,798,706 and 4,801,431. The previously known devices are limited in their performance and unable to satisfy the needs for automated, high precision immunohistology.

It is an object of this invention to provide a device which provides more rapid, reliable and more reproducible results than standard methods; can perform any standard immunochemical assay including assays relying on immunofluorescence, indirect immunoassay procedures, peroxidase anti-peroxidase methods, or avidin-biotin technology; preforms all steps of the immunohistochemical assay irrespective of complexity or their order, at the time and temperature, and in the environment needed; and is cost effective in terms of equipment, reagent and labor costs.

DISCLOSURE OF THE INVENTION

The automated biological processing apparatus of this invention comprises a reagent carousel cooperating with a sample support carousel to apply a sequence of preselected reagents to each of the samples with interposed mixing, incubating, and rinsing steps cooperating therewith. The slide support carousel has a plurality of slide supports thereon and drive means engaging the slide support carousel for consecutively positioning each of a plurality of slide supports in a reagent receiving zone. The reagent carousel has a plurality of reagent container supports thereon and drive means engaging the reagent carousel for rotating this carousel and positioning a preselected reagent container support and associated reagent container in a reagent supply zone. The apparatus has a reagent delivery actuator means positioned for engaging a reagent container positioned on a container support in the reagent supply zone and initiating reagent delivery from the reagent container to a slide supported on a slide support in the reagent receiving zone.

The apparatus preferably has bar code readers positioned to read bar codes on the sample containers or slides and on the reagent containers. Each of the carousels have homing systems containing a detectable component and a proximity detector therefor for indexing the position of the reagent containers and slides.

One particular advantageous feature of the present invention is that by employing a computer control arrangement to

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control the positioning of the reagent and slide support carousel, different reagent treatments can be individually performed for each of the various tissue samples by appropriate programming of the apparatus. Additionally, the provision of the bar code readers permits tracking of each of the tissue samples as well as a record of the reagents applied thereto.

The apparatus preferably has a heating chamber means surrounding the slide support carousel for heating slides supported thereon to a predetermined temperature. The heating chamber means includes a hot gas manifold having a plurality of hot gas outlets positioned above the slide supports. The heating chamber means includes a temperature sensor and a hot gas control means connected to the temperature sensor for increasing heat supplied to gas flowing through the manifold and for increasing the hot gas flow rate if further heat is required to maintain the heating chamber at a preselected temperature. The temperature sensor is a thermistor, the tip thereof being enclosed in a heat sensitivity reducing jacket. The hot gas control system includes two heating components with separate controls and a speed control for the hot gas fan.

The drive means engaging the slide support carousel is also a means for consecutively positioning each of a plurality of slide supports at rinse zone, an evaporation control liquid and reagent receiving zone, a vortex mixing zone including vortex mixing means, and an incubation zone formed by the heating chamber means.

According to a first embodiment of the rinse zone, rinse spray means are positioned adjacent to the rinse zone for applying pulses of rinse liquid to the surface of each of the slides positioned in the rinse zone. The apparatus slide supports are, according to this first embodiment of the rinse zone, pivotally mounted for pivotal motion from a horizontal slide incubation position to a tilted slide draining position following each pulse of rinse liquid.

According to a second embodiment of the rinse zone, first and second rinse spray means are respectively positioned only at the beginning and end of the rinse zone, so as to be spaced from one another. The first rinse spray means deposits a layer of rinse liquid onto a slide upon entering the rinse zone and the second spray means, after a predetermined waiting period, uses pulsed streams of rinse liquid, alternately directed at the longitudinal edges of the slides, to knock the previously deposited layer of rinse liquid off of the slide as the slide exits the rinse zone. According to this second embodiment of the rinse zone, the apparatus slide supports are stationary, a jet drain being provided at, for example, the end of the rinse zone, which directs a stream of fluid, such as, for example, air or the like, over the slide to drain any remaining rinse liquid off of the slide surface.

The apparatus preferably has a volumetric pump means, and a reagent delivery actuator means positioned for activating the volumetric pump means, thereby effecting delivery of reagent from a reagent container by the volumetric pump to the reagent delivery zone. An evaporation inhibitor liquid application means is positioned adjacent the reagent delivery zone.

Vortex agitation means are positioned adjacent the agitation zone for stirring reactants on a slide supported in the vortex agitation zone.

The pivoting slide support has distal and proximal ends, the distal end having raised terminal and lateral distal guide tabs with guide termini. The proximal end has first and second lateral guide tabs with opposed slide engaging surfaces for engaging and holding the lateral edges of a slide.

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The guide termini are lower than the upper slide surface plane. In this embodiment of the slide support, the slide support surface is tipped or pivoted by a tipper to drain rinse liquid from the surface of the slide.

The stationary slide support has a slide support platform at a proximal end and a slide support post at a distal end thereof. The distal end also has raised lateral distal guide tabs with guide termini between which a slide is positioned. The slide support platform at the proximal end has a guide edge and a slide clamping arrangement for clamping a slide to the support platform without interfering with the reading operation of the bar code reader. The distal guide termini are lower than the upper slide surface plane to prevent wick-off of liquid on the slide surface. In this embodiment, rinse liquid is drained from the surface of the slide employing a jet drain which directs a stream of fluid, i.e., gas or liquid, over the slide surface.

An improved biochemical method of this invention with increased sample dehydration protection comprises carrying out a biochemical reaction under a layer of evaporation inhibiting liquid. The improvement comprises (a) covering the sample with an aqueous surface layer by applying an aqueous solution to a planar support surface adjacent a biological sample mounted thereon; and (b) covering the aqueous surface layer with an evaporation inhibiting liquid layer by applying the evaporation inhibiting liquid to the planar support surface adjacent the biological sample in an amount sufficient to form a continuous layer of evaporation inhibiting liquid over the sample. The evaporation inhibiting liquid is substantially water-insoluble, substantially water-immiscible and substantially non-viscous; has a specific gravity less than water, and a boiling point above 50° C.; and is devoid of chemical characteristics which would significantly interfere with biochemical reactions carried out on the sample. The biological sample can then be optionally treated (c) with an aqueous reagent solution by applying the reagent solution to the planar support surface adjacent the biological sample. The reagent solution flows to the biological sample under the evaporation inhibiting liquid layer, and the sample is continuously protected from dehydration by the evaporation inhibiting layer.

In another aspect of this invention, the reagent solution is stirred on the surface of the biological sample by applying at least one gas stream to an area of the surface of the evaporation inhibiting liquid layer between the center of the evaporation inhibiting liquid layer and the edge of the planar support surface, the gas stream having a central axis forming an acute angle with the planar support surface. According to one embodiment of the present invention, the reagent solution is preferable stirred by a vortex formed by applying two off-center gas streams, flowing in opposite directions, to the surface of the evaporation inhibiting liquid layer. According to a further embodiment of the present invention, the reagent solution is stirred by a vortex formed by applying a single gas stream along a longitudinal edge of the slide, the gas stream originating from the distal edge of the slide.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a left front, isometric view of the automated immunostaining apparatus according to a first embodiment of this invention which employs a tipper rinse method, with the cabinet shell removed.

FIG. 2 is an exploded right front isometric view of the apparatus shown in FIG. 1.

FIG. 3 is a partial exploded left front isometric view of the apparatus shown in FIG. 1.

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FIG. 4 is a partial exploded right rear isometric view of the apparatus shown in FIG. 1.

FIG. 5 is a top view of a pivotally mounted slide support.

FIG. 6 is an isometric view of the underside of the slide support component.

FIG. 7 is a side view of the pivotally mounted slide support of FIG. 5 showing the tipper and mounting details.

FIG. 8 is an isometric view of the mounted slide support of FIG. 7 in the untipped position.

FIG. 9 is an isometric view of the mounted slide support of FIG. 7 in the tipped position.

FIG. 10 is a distal end view of the mounted slide support in the tipped position.

FIG. 11 is a fragmentary top view of the slide support carousel showing details of the slide treatment stations.

FIG. 12 is a schematic cross-sectional view of a rinse station taken along the line A—A in FIG. 11, showing details of rinse liquid flow on a slide.

FIG. 13 is a top schematic view of the rinse stations showing details of the rinse liquid distribution on slides being treated therein.

FIG. 14 is an isometric view of the slide treatment bar code reading, rinse, reagent receiving and vortex mixing stations.

FIG. 15 is a schematic, fragmentary cross-sectional view of the evaporation inhibiting liquid and reagent receiving station, taken along the line B—B in FIG. 11.

FIG. 16 is a cross-sectional view of the vortex mixing assembly, taken along the line C—C in FIG. 11.

FIG. 17 is a top schematic view of the vortex mixing zone, showing details of the vortex mixing action.

FIGS. 18A—C are schematic representational cross-sectional views of a slide following the rinse liquid (FIG. 18A), evaporation inhibitor (FIG. 18B) and reagent application (FIG. 18C) steps.

FIGS. 19A—19B are cross-sectional views of respective alternative embodiments of a rinse liquid container and associated heating components.

FIG. 20A is a bottom, isometric view of one embodiment of a reagent container support tray.

FIGS. 20B—20C are side sectional views of a further embodiment of the reagent container support tray.

FIG. 21 is a fragmentary cross-sectional view taken along the line D—D in FIG. 11 showing the slide carousel metal proximity sensor indexing system of this invention.

FIG. 22 is a schematic view of the pneumatic system of the automated immunostaining apparatus of this invention.

FIG. 23 is a schematic drawing of the 120 volt AC power distribution in the apparatus of this invention.

FIG. 24 is a schematic drawing of the DC power distribution in the apparatus of this invention.

FIG. 25 is a schematic drawing of a first portion of the computer digital I/O system in the apparatus of this invention.

FIG. 26 is a schematic drawing of a second portion of the computer digital I/O system in the apparatus of this invention.

FIG. 27 is a schematic drawing of the computer serial and floppy disk I/O system in the apparatus of this invention.

FIG. 28 is a further embodiment of the intermediate section of the apparatus of this invention which dispenses with the tipper rinse method.

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FIGS. 29A—29B are top and side views respective an alternative embodiment of the slide support for use with the embodiment of FIG. 28.

FIG. 30A is a side, isometric view of one embodiment of a single wash block nozzle for use with the embodiment of FIG. 28.

FIG. 30B is a side, cross-sectional view of the single wash block nozzle of FIG. 30A.

FIG. 31 is a side, isometric view of one embodiment of a dual wash block nozzle for use with the embodiment of FIG. 28.

FIG. 32 is a top view of a further embodiment of the vortex mixers for use with the embodiment of FIG. 28.

FIGS. 33A—33B are side and front views respectively of bar code cleaning arrangement for use with the embodiment of FIG. 28.

FIG. 34 is a schematic of a jet drain for draining liquid from an upper surface of a slide.

BEST MODE FOR CARRYING OUT THE INVENTION

The automated immunostaining apparatus of this invention preforms all steps of immunohistochemical and in situ DNA assays irrespective of complexity or their order, at the time and temperature, and in the environment needed. Specially prepared slides containing a bar code identifier and a mounted tissue section are placed in special support on a carousel, subjected to a preprogrammed sequence of reactions, and are removed from the carousel, ready for coverslipping and histological examination. For purposes of clarity of the following description of the apparatus of this invention and not by way of limitation, the apparatus will be described in terms of immunohistochemical processes.

FIG. 1 is a front right, isometric view of the automated immunostaining apparatus of this invention, with the cabinet shell removed. Liquid and air supply tubing and electrical wiring connecting the respective components are conventional, well known in the art, and are omitted from the drawings for purposes of clarity. The apparatus has an upper section 2, intermediate section 4 and lower section 6. In the upper section 2, reagent bottle support carousel 10 is mounted for rotation about its central axis 7 on upper support plate 8. Reagent bottles 12 required for the immunohistochemical reactions to be conducted during slide treatment cycle are supported by the carousel 10, mounted in reagent bottle receptors 11. These receptors 11 are configured to receive volumetric pump outlet tube 307, shown in detail in FIG. 15. The receptors 11 are preferably equally spaced in a circular pattern axially concentric with the carousel axis 7. The number of receptors 11 provided should be sufficient to accommodate the number of different reagent bottles 12 required for a cycle or series of cycles. Twenty-five receptors 11 are shown, but the number can be smaller or greater, and the diameter of the carousel 10 can be increased to accept a larger number of reagent bottles 12. The carousel 10 is rotated by the stepper motor 14 drive belt 16 to a position placing a selected reagent bottle 12 in the reagent deliver position under the air cylinder reagent delivery actuator 18 over a slide to be treated with reagent. Reagent tray motor driver 20 is connected to stepper motor 14.

The intermediate section 4 comprises support plate 22 upon which the slide support carousel 24 is rotatably mounted. The carousel 24 supports slide supports 26. Heated air supply chamber 28 communicates with the heated air

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supply manifold **30** supported on the underside of plate **8** and lid heated air supply manifold **31** mounted on the upper plate **8** by hinged supports **33**. The support plate **22** also supports the conventional computer board **32**, LCD display **34**, disk drive **35** and computer **36** used to operate the apparatus. Air pressure regulator **38**, as best seen in FIG. 2, regulates the pressure of air delivered to the evaporation inhibitor and rinse liquid delivery systems described in FIG. 22.

The lower section **6** includes support plate **40** upon which are supported accessories such as power supply filter **42** and hot water supply **44**.

FIG. 2, FIG. 3 and FIG. 4 are exploded right front, left front and right rear isometric views of the apparatus shown in FIG. 1. Tipper air cylinders **46** are positioned on support plate **8**. These cylinders are aligned to actuate a tipper cam surface **148** against a slide support tab surface **112** shown in detail in FIGS. 8, 9 and 10.

In the intermediate section **4**, the stepper motor **48** rotates the slide support carousel **24**, engaging drive belt **25** (FIGS. 3 and 4) engaging the perimeter of the slide support carousel **24**. Splash guard **50** is a wall which surrounds the sides, back and part of the front of the carousel **24**, defines the heating zone and contains the liquid spray and droplets produced in the processing. It extends upward from the intermediate plate **22** to a position adjacent the upper plate **8**, leaving an air flow gap between the upper edge of the splash guard **50** and the underside of the plate **8**. Mounted on the underside of upper support plate **8** above the carousel **24** and within the perimeter of the splash guard **50** is the heated gas supply manifold **30** (FIG. 2). Heated air is directed downward and over the slide supports **26** by holes **336** (FIG. 15) in the manifold **30**. The heated air then passes upward over the top of the splash guard **50** and exits the device. Extending upward through central opening **52** of carousel **24** into the heated air supply chamber **28** is the fan shroud **54** and axially positioned fan **56**. The fan **56** is positioned over air vents **57** in the bottom plate **22**. The annular waste liquid sump **58** surrounds the shroud **54**, below liquid outlet ports **292** (FIG. 14), and is supported on the bottom of plate **22**. The waste reagent and rinse liquids are collected in the sump bottom (not shown).

Rinse and liquid coverslip spray blocks **60** are supplied with liquid through conventional solenoid valves **62**.

Temperature controller **66**, mounted on support plate **22**, controls the heat energy supplied to the heated water container **44**. Temperature controllers **68** and **70**, mounted on support plate **40** (FIG. 4), control the temperature of the air in the heated air supply chamber **28** by controlling energy supplied to respective annular heater elements **331** and **332** (FIG. 15). Slide carousel stepper motor driver **72** and relay **74** operate stepper motor **48**. Power supplies **76** and **78** provide power to the stepper motors and control systems. Air compressor **80** supplies air to the air filter **82** and air pressure regulators **38**, **64** and **86**.

FIG. 5 is a top view of a first embodiment of a mounted slide support **26** with slide edges **100** and **101** represented by dashed lines. The slide support **26** has a support plate **102** with a distal end **103** and a proximal end **104**. The distal end **103** has a raised terminal guide end tab **106** and two lateral guide tabs **108** and **110** with the upper edges constituting guide tab termini. The distance between the upper surface of the slide support **26** and the guide tab termini (the elevation above the upper surface) is less than the thickness of a conventional microscope slide. The proximal end **104** of the

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slide support **26** has opposed lateral guides **112** and **114** for engaging the lateral edges of a slide and a terminal end tab **115** for engaging the proximal end of a slide. The proximal end **104** of the slide support **26** has an inflexible support portion **116** providing a lateral edge **120** and a flexible arm **118** including a lateral edge **122** positioned such that lateral edges **120** and **122** oppose one another. The distance between the slide edge engaging surfaces **111** and **113** of the guide tabs **112** and **114** is less than the width of a slide to be supported on the slide support **26**. A standard slide has a width of 1 inch or 25 mm, and the preferred distance between the slide edge engaging surfaces **111**, **113** of the tabs **112**, **114** for supporting a standard slide is from 20 to 24 mm. The flexure of arm **118** permits positioning of the slide between the lateral guide tabs and terminal end tabs **106**, **115**. The distance between the opposing tab surfaces **111** and **113** causes the slide support **26** to apply a positive pressure on the edges of a slide, retaining the slide securely on the slide support **26** during the tilting and other processing steps. The upper surface of the support plate **102** is preferably planar and smooth so the wet slide rests closely on the surface **102**, and surface tension will resist vertical movement of the slide from the support surface **102**.

FIG. 6 is an isometric view of the underside of the slide support **26**. The inflexible portion **116** has an integral pivot support **124** which reinforces the inflexible portion **116** to prevent flexure. The flexible arm **118** has sufficient depth or thickness to limit the flexural movement of the arm **118** to a horizontal direction. This insures effective cooperation and pressure between the guide tab **112** on the inflexible portion **116** and the guide tab **114** on the flexible arm **118** to assist in retaining the slide in place on the slide support **26** during the tipping operation described in detail hereinafter.

FIG. 7 is a side view of a mounted slide support showing the tipper and mounting details. The upper pivot support **124** is pivotally mounted on the lower pivot support **126**. Lower pivot support **126** has upward extending projections **128** and **130** which engage the ends **132** and **134** of the upper pivot support **124**. Pivot pin **136** extends through an axially aligned hole in projection **128** into an axially aligned receptor hole **138** (FIG. 6) in the opposing end **132** of the upper pivot support **124**. At the opposite end, axially concentric with pivot pin **136**, pivot pin **140** extends through a hole in projection **128** (not shown) into a respective receptor hole in the opposing end **134** of the upper pivot support **124**. The slide support **102** is thus mounted for pivotal motion around the common pivot axis of the pins **136** and **140**. Bias spring **142** is supported on pin **134**, one end **141** pressing against the lower abutment surface **143** of the inflexible support portion **116**, and the other end **144** bearing against spring stop groove **145** in the spring stop **146**. The tip **148** of tipper **150** is positioned above the upper surface of guide tab **112** when the slides are positioned in a rinse station, described in greater detail hereinafter with respect to FIG. 13.

The pivot pins **136** and **140** support the upper surface of the slide support **102** at a small angle 'a' from the horizontal plane to aid liquid flow toward the distal end **103** during treatment. Angle 'a' is preferably in the range of from 0.3 to 1.0°. The upper surface **151** of the inflexible support portion **116** and the upper slide surface **152** (dotted line) supported thereon are thus maintained at a slight incline from the horizontal plane downward toward the distal end **103** of the slide support **26**.

FIG. 8 is an isometric view of a slide (dashed lines) mounted on slide support **26** in the untipped position, FIG. 9 is an isometric view of the mounted slide support **26** in the tipped position, and FIG. 10 is a distal end view of the

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mounted slide support **26** in the tipped position. Vertically downward pressure of the tipper tip **148** against the upper guide tab surface **154** of guide tab **112** rotates the support plate **102** about the pivot axis **156** defined by the pivot pins **136** and **140**. The pivot axis **156** (FIG. 5) preferably lies in a vertical plane through the midpoint of distal end **103** and the left edge proximal end **104** of the slide support **26**. The tipping action tilts the slide surface to an angle 'c' of approximately 600 from the vertical (FIG. 10). It sharply lowers distal corner **158** and sharply raises proximal corner **160**, breaking the liquid meniscus on the slide surface and directing the liquid flow **159** to the corner **158** and off the surface of the slide into drain hole **292**. The pivotal movement increases the pressure of the spring **142** against spring stop groove **145**, and as the tipper **150** is raised, the slide support **25** returns to its original position. The slide support return pivot motion is terminated when distal corner **162** of the support plate **102** abuts stop surface **164** of the lower pivot support **126**.

FIG. 11 a fragmentary top view of the slide support carousel **24** showing details of the various slide treatment stations. Rinse nozzle blocks **200**, **202** and **204** and the adjacent respective slides **206**, **208** and **210** define successive rinse zones, details of which are shown in FIGS. 12-14. Evaporation inhibitor liquid application block **212** and the adjacent slide **214** define the evaporation inhibitor and reagent application zone, details of which are shown in FIG. 15. Air cylinder reagent delivery actuator **18**, supported by support arm **216**, contacts reagent bottle **218**, directly over slide **214**. Vortex mixer air jet blocks **220**, **222** and **224** are positioned adjacent slides **226** and **228** in the agitation zone, details of which are shown in FIGS. 16 and 17. The hanger **352** is mounted on the tip of blocks **220** and **222** and supports suspended block **224**. Pressurized air is delivered to block **224** by conduit **358**. As the slide support carousel **24** positions each slide for successive treatment in the rinse zones, evaporation inhibitor and reagent application zone, and agitation zones (counterclockwise movement of the carousel), the tissue sections on each slide are first rinsed and then covered with evaporation inhibitor. Reagent is applied from a preselected reagent bottle to the tissue through the evaporation inhibitor layer, and the reagent is agitated through the evaporator inhibitor layer by the vortex mixer. Each slide then is moved around the incubation zone, a circular path traveled by the slide support carousel **24**, heated with hot air from the heated air manifold **30**, and the reagent reacts with the sample. As the carousel **24** continues to increment around the circle, each slide is returned to the rinse stations, etc., for application of the next reagent required in the reaction. This entirely automated process continues until the desired reactions are completed.

Bar code reader **231** (FIG. 14) above slide **205** reads a slide bar code **233** (FIGS. 13 and 17) on each slide. The slide bar codes **233** identifies the slide sample and the particular immunohistochemical process required for that sample. This information is fed into the computer and correlated with the indexed position of that slide with respect to "home", to control the sequence of reagent chemicals to be applied to that slide in the reagent application zone.

FIG. 12 is a schematic cross-sectional view of a rinse station taken along the line A—A in FIG. 10, showing details of rinse liquid flow on a slide. Rinse block **200** mounted on plate **22** has a heated rinse liquid supply channel **230** communicating with rinse liquid nozzle **232**. The slide **234** has a sloping surface at an angle 'a', being supported on the sloping surface of the slide support **102**. The slide **234** has a rinse liquid impact zone **236** adjacent the proximal end **104**

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between the bar code **233** and the sample **238**. The impact zone **236** is at a higher elevation than the tissue section **238** supported adjacent the distal end **103**. The nozzle axis **240** has an angle 'b' which directs liquid against the slide surface impact zone **236**. The impact zone **236** is above the tissue section **238** on the sloped surface of slide **240**, and the rinse liquid stream **242** flows across the upper surface of the tissue section **238** toward the distal end **103**. The angle 'b' preferably has an angle of from 15 to 35°, and the distance between the exit of nozzle **232** and the slide **124** is selected to direct the rinse liquid precisely on the impact zone **236**, avoiding disturbance of the fragile tissue section **238**.

The slide support carousel **24** is rotated above the plate **22**, the outer periphery being supported by low friction slide bearings **244** arrayed in an axially concentric circular path on plate **22** under the outer periphery of carousel **24**.

FIG. 13 is a top schematic view of one embodiment of the rinse stations showing details of the rinse liquid distribution on slides being rinsed therein. Slides **234**, **246**, and **248** are positioned in the path of heated rinse solutions (dotted lines) from rinse station blocks **200**, **202** and **204**. Fragile tissue sections **238**, **250** and **252** are positioned adjacent the distal end of the slides. The rinse liquid impact zones **236**, **254** and **256** are positioned between the tissue sections and the proximal ends of the slides, to avoid direct impact of the liquid jets from the rinse block nozzles. The rinse nozzles on each block are preferably 11.5 mm apart. Rinse block **200** has right offset nozzles **232** and **258** (offset 2 mm to the right of center) supplied by channel **230** connected to supply tubing **260**. This directs the rinse fluid toward the right surface of the slide, effecting a transverse flow path across the tissue section **238** to the distal end drain corner **158**. Rinse block **202** has symmetrical nozzles **262** and **264** supplied by channel **266** connected to supply tubing **268**. The symmetrical nozzle configuration effects a central flow path across the tissue section **250**. Rinse block **204** has left offset nozzles **270** and **272** (offset 2 mm to the left of center) supplied by channel **274** connected to supply tubing **276**. The left offset nozzles **270** and **272** direct a rinse flow path down the left side of the tissue section **252**. The nozzle patterns provide effective rinse solution flow distribution across all portions of the tissue section surface as the slide is treated in each successive rinse section.

FIG. 14 is an isometric view of the rinse stations, a evaporation inhibiting liquid and reagent application station, and agitation stations, showing details of the slide tipping action in the rinse sections. Tipper air cylinders **46** (FIGS. 3 and 4) comprises three conventional air cylinders **278**, **280** and **282** with internal pressurized air activated pistons or equivalent actuators. Pressurized air delivery to the cylinders causes respective tipper tips **148**, **284** and **286** to move downward, pressing against respective slide support tabs **112**, **288** and **290**. Three tipper positions are shown to illustrate the action thereof. Tipper tip **148** is shown in the fully withdrawn or resting position, and slide **206** is in the rinse solution receiving position. After application of heated rinse solution, the tipper descends through an intermediate position shown by tipper tip **284** and slide support **208**, to the drain position shown by tipper tip **286** and slide support **210**. Liquid drains from the left distal corner (lowest corner) into a drain hole **292**.

In each rinse station, the sample is treated with a repeated, preferably at least seven, rinse cycles. Each rinse cycle comprises application of approximately 500 μ L of heated rinse solution in a short pulse (120 msec) to the slide, followed by tipping the slide to drain away the rinse solution. An estimated 150 μ L of liquid remains on the slide after

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draining. These rinse cycles are repeated in each rinse station. The short rinse pulse followed by draining prevents the formation of an equilibrium solute boundary layer and greatly increases the rinse efficiency, overcoming the boundary problems present in the prior art rinse methods. Assuming that 150 μ L of rinse solution is left after each draining step, a 23 percent dilution is achieved with each rinse cycle. Thus the effective dilution in the combination of the three rinse stations is estimated to be 0.2 parts per trillion, many orders of magnitude more effective than prior art, biochemical rinse procedures. This greatly increases the sensitivity of the immunohistological process.

FIG. 15 is a schematic, fragmentary cross-sectional view of the evaporation inhibiting liquid and reagent application station, taken along the line B—B in FIG. 11. Evaporation inhibitor liquid distributor block 212 has a supply channel 293 and outlet nozzles 294.

The evaporation inhibiting liquid is substantially water-insoluble, substantially water-immiscible and substantially thin or non-viscous. It has a specific gravity less than water, and a boiling point above the process temperature, preferably above 100° C. It should be devoid of chemical characteristics which would significantly interfere with biochemical reactions carried out on the sample, that is, the reactions taking place between the reagents and tissue sample on the slide. Preferred evaporation inhibiting liquids are hydrocarbons, optimally non-aromatic saturated hydrocarbons, having from 9 to 18 carbons, most optimally having about 10 to 14 carbon atoms.

A small quantity of evaporation inhibitor liquid is directed by nozzle 294 in an inhibitor liquid stream 296 to an impact zone 298 on the slide between the tissue sample 238 and the proximal end 100 of the slide, so that the tissue sample is not disturbed. The evaporation inhibitor liquid flows across the surface of the water layer on the wetted tissue, forming a thin evaporation inhibiting film 299 over the aqueous layer which usually covers most of the upper surface of the slide. The tissue is now ready for application of reagent.

The reagent delivery combination includes a conventional air cylinder 18 or equivalent actuator having an internal pressurized air activated piston. It is supplied with pressurized air by tubing 300. Air cylinder 18 is supported by plate 216 and post 302 mounted on upper plate 8. Delivery of pressurized air to the cylinder 18 causes rod 304 and its attached foot 306 to move downward against a reagent container 12 positioned in the reagent delivery zone. Downward movement of reagent container 12 causes emission of a precise volume of reagent liquid 310. Suitable volumetric pumps are available from S. A. Valois and are described in U.S. Pat. No. 4,245,967 and French patent 2,528,122.

The reagent carousel support 314 is the drive plate which supports the reagent bottle carousel 10 and rotates it about its axis to place a predetermined reagent bottle 12 in the reagent delivery zone. An axially concentric circular array of low friction slide bearings 316, mounted on the upper plate 8, are positioned under the outer edge of the reagent support carousel.

The predetermined volume of aqueous reagent 310 impacts the evaporation inhibitor surface film between the impact zone 298 and the upper edge of the tissue sample 299, passing through the film to the aqueous layer beneath the film and reaching the slide surface. The reagent then flows across the tissue sample 238 under the covering film of evaporation inhibiting liquid 299. In this sequence, immediately after leaving the rinse stations, the slide is covered with the protective film to prevent any dehydration of the

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tissue sample 299. The reagent solution is then applied to the protected tissue. Dehydration of the tissue section would irreversibly alter its physical and chemical characteristics and impair the immunohistochemical reactions. Dehydration is a constant hazard because of the constant flow of heated air over the slides required to maintain them at the desired temperature. The heated air temperature is determined by the requirements of the biochemical processes required by the process. It is slightly above 40° C., preferably about 45° C., for immunochemical reactions and can be as high as from 93 to 97° C. for in situ DNA hybridization reactions.

FIG. 15 also shows detailed elements of the heated air supply chamber 28 shown in FIG. 1. Air is moved upward into the central intake manifold chamber 330 and through annular heating coils 331 and 332 mounted on annular air passageway plate 333, to heat the air to a temperature slightly above 40° C., preferably about 45° C. A higher temperature can be provided as needed for in situ DNA hybridization procedures. The heated air passes through the outlet manifold chamber 334 and out the outlet passageways 336 in the lower plate 338. Annular, axially concentric inner and outer heated air flow control curtains 340 and 342 direct the heated air downward over the slide surface. The reagent 310 falls through manifold passageway 344 to the slide surface.

The air temperature is monitored by heat sensor 345 positioned in the path of the heated air. A preferred heat sensor is a thermistor encased in a heat sensitivity adjusting jacket 347 which reduces the sensitivity of the thermocouple and approximates the thermal mass of the slides.

A reagent bar code reader 346 can be mounted on post 302, positioned to scan a reagent bar code 348 on the reagent bottle 12. Bar code 348 identifies the contents of the reagent bottle. At the beginning of a slide treatment operation, the reagent carousel 10 is rotated past the bar code reader 346, and the bar code 348 on each reagent bottle 12 is scanned. The scanned information is fed to the computer and correlated with the indexed position of the reagent carousel 10. This information is used to rotate the reagent carousel 10 to place the correct reagent bottle 12 in the application zone for each slide treatment step for each slide.

FIG. 16 is a cross-sectional view of one embodiment of the vortex mixing assembly, taken along the line C—C in FIG. 11. Outer vortex jet block 222, mounted on plate 22, has an pressurized air supply channel 350 and nozzle 351. Nozzle hanger 352 is mounted on the top of vortex block 22 and supports suspended inner vortex air jet nozzle block 224. Channel 354 supplies nozzle 355 in block 224 with pressurized air. Nozzles 351 and 355 have central axes which form angles 'd' and 'e' of from 5 to 15° with the horizontal, directing air jets 356 and 357 toward the slide surface at the corresponding acute angles.

FIG. 17 is a top schematic view of the vortex mixing zone, showing details of the vortex mixing action. Pressurized air is supplied to the nozzle channels 350 and 354 by channel 358. The reagent solution covered by a layer 360 of evaporation inhibiting liquid 360 is stirred on the surface of the biological sample by applying at least one gas stream 356 or 357 to an area of the surface of the evaporation inhibiting liquid layer 360 between the center of the evaporation inhibiting layer 360 and the edge of the planar support surface 361 or 362 of the slide 228. The gas stream impacts the surface of the evaporation liquid surface layer 360 and moves the underlying reagent solution in a circular path on the tissue section. Preferably, the reagent solution is stirred

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on the surface of the biological sample by a vortex formed by applying two gas streams **356** and **347**. Stream **356** is directed against a area **363** of the surface of the evaporation inhibiting liquid layer between the center of the evaporation inhibiting layer and the slide edge **361**. Stream **357**, in a direction opposite to the direction of stream **356**, is directed against an area **364** of the surface of the evaporation inhibiting liquid layer between the center of the evaporation inhibiting layer and the slide edge **362**. Although this method is shown with respect to an evaporation liquid inhibitor covered reagent layer, it will be readily evident that it can be applied to gently stir any liquid layer overlying a fragile substance.

FIG. **18** is a schematic representational cross-sectional view of a slide **370** following the rinse liquid, evaporation inhibitor and reagent application steps. Following the rinse stages (Stage A), the tissue section **371** mounted on slide **370** is covered with a thin residual aqueous layer **372**. Following application of the evaporation inhibitor liquid (Stage B), the aqueous layer **372** and tissue section **371** is entirely covered by a layer **373** of the evaporation inhibitor liquid. Aqueous reagent **374**, applied to the slide, flows under the evaporation inhibitor layer **373** to cover the tissue section. In the vortex mixing section (Stage C), air jets directed against the surface of the evaporation inhibitor liquid **373** move it and the reagent solution **374** thereunder in a swirling or stirring action on the surface of the fragile tissue section. This gentle stirring achieves increased interaction of reagent with the tissue section while reserving the tissue from dehydration or other damage from the air jets.

FIG. **19A** is a cross-sectional view of one embodiment of a rinse liquid container and associated heating components. The rinse liquid applied to the surface of the slides by rinse blocks **200**, **202** and **204** should have a temperature above 40° C. and is preferably about 45° C. The elevated temperature is critical for the immunochemical reactions. The rinse liquid is supplied by the hot water supply **44**. The hot water supply **44** comprises an inner container of an inert material having a low coefficient of expansion such as a pyrex bottle **382** having a threaded neck **384** to which a cap **386** is attached by threads. The container **382** is surrounded by an insulating jacket **388** of suitable insulation material such as a fiberglass layer. Between the insulating jacket **388** and the bottle **382** is a heating jacket **390** with electrical power leads **392**. A suitable heating jacket is a thick sheet of silastic rubber (polysiloxane) with embedded resistance heating coils having a combined heating value of about 180 watts. A conventional safety thermostat **394**, connected to the elements of the heating jacket, is also provided between the insulating jacket **388** and bottle **382**. The safety thermostat prevents the rinse liquid temperature from exceeding a preset value, preferably about 50° C. A thermistor temperature sensor **391** with leads **393** extends through the cap **386** into the upper zone of the bottle **382**. An liquid inlet tube **394** extends through the cap **386** to the bottom of the neck **384**, and an outlet tube **396** extends through the cap **386** to the bottom of the bottle **382**.

This unique configuration provides a highly uniform liquid output temperature. The colder water entering through the inlet tube **394**, being more dense than the heated liquid in the bottle, sinks downward past the heated container walls and is heated. The displaced liquid rises upward in the container. This stirring motion thoroughly mixes the liquid without the need for an agitator, producing a highly uniform outlet liquid temperature. Thermistor **391** constantly monitors the liquid temperature, providing a signal to the control system which is used to determine when the heating elements in jacket **390** should be energized.

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FIG. **19B** illustrates an alternative embodiment of the rinse liquid container and associated heating components of the present which is similar to the structure illustrated by FIG. **19A** except that the inlet tube **394** of the embodiment of FIG. **19** functions as an outlet tube **394A** and outlet tube **396** of the embodiment of FIG. **19** functions as an inlet tube **396A**, i.e., the inlet and outlet lines have been reversed. This arrangement prevents the build up of air or gas in the bottle **384**. Additionally, the inlet tube **396A** has been provided with perforations **396B** for obtaining mixing as the bottle **384** is replenished with liquid.

FIG. **20A** is a bottom, isometric view of one embodiment of a reagent container support carousel **10**. According to this embodiment, the reagent container carousel **10** has feet **800**, **801** and **802** which rest in respective matching recesses in the reagent carousel support **314** (FIG. **15**) in only one position. This insures that the reagent carousel **10A** and the reagent bottle receptors **11** are always positioned in predetermined orientation on the carousel support **314**.

The feet **800**, **801** and **802** also function as supporting feet when the reagent support carousel **10** is removed. Refrigeration of the reagents is often required during their storage. The reagent container carousel **10**, with the reagent bottles supported thereon, can be lifted from the carousel support **314** and placed in a refrigerator, supported by the feet **800**, **801** and **802**.

Indexing metal homing block **803** is mounted on the reagent container carousel **10** and rotates with the carousel **10**. A conventional metal proximity detector (not shown) is mounted on the upper plate **8** at an position which places it adjacent the rotational path of the homing block. A change in electrical signal from the proximity detector indicates that the metal homing block is in the 'home' position adjacent the block.

FIG. **20B** is an alternative embodiment of a reagent support carousel **10A** and associated carousel support **314A** wherein a handle **804** has been provided to assist in the removal and replacement of the reagent support carousel **10A** as described above. In this embodiment, the carousel **10A** is provided with a plurality of feet **800A**, for example, five feet, which are substantially cylindrical elements with beveled edges **805**, and fit into corresponding and matching circular openings **802A**, formed in the associated carousel support **314A**. The feet **800A** and opening **802A** are positioned so that the carousel **10A** will fit into the support **314A** in only one position such that the carousel **10A** is always positioned in a predetermined orientation on the support **314A**. The support **314A** is provided with a central hub **806** which is received in a central opening **807** formed in the carousel **10A**, the hub being provided with beveled edges **808**. Engagement of the carousel **10A** and the support **314A** is best seen in FIG. **20C**. Except for the above described differences, the carousel **10A** and the support **314A** are the same as previously described.

FIG. **21** is a fragmentary cross-sectional view taken along the line D—D in FIG. **11**. Indexing block **229** is a metal block. Proximity sensor **610** is supported on the underside of plate **22** by bracket **611**. The proximity sensor **610** emits an electrical signal through leads **612** which changes when the metal block **229** is positioned in the 'home' position immediately above the sensor.

The homing systems of the reagent carousel **10** and slide support carousel **24** operate in a similar manner. Presence of an indexing block adjacent the sensor produces a signal indicating that the carousel is in a "home" position, and provides a reference for subsequent indexed movements of

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the respective stepper motor drive and subsequent indexed movements of the respective carousel.

FIG. 22 is a schematic view of the pneumatic system of the automated immunostaining apparatus of this invention. The air supply for the system is supplied by air compressor **80** and air filter **82**. The output line **400** from the air filter **82** is connected to the input port of air pressure regulator **86** where it is regulated to a constant output pressure of about 25 psi. Diaphragm pressure switch **402** communicates with the air pressure regulator **86** outlet line **403** through line **404**. Diaphragm pressure switch **402** closes the system circuit breaker **406** when the pressure in line **404** is at least 22 psi. Failure of the air compressor and resulting drop in line pressure automatically deactivates the system.

The air output branch line **408** lead is connected by line **410** with tipper air cylinder three way control solenoid valve **412**. When in an "open" position, solenoid valve **412** provides communication between input line and cylinder **278**. This permits pressurized air to pass from line **410** to air cylinder **278**, thus pressing tipper tip **148** (FIG. 14) against the respective slide support tab **112** and tipping the slide support **206**. When solenoid valve **412** returns to the vent position, the air cylinder **278** communicates with atmosphere, permitting the air cylinder **278** to return to its resting position. Tipper tip **148** then rises to its resting position, allowing the slide support to also return to its horizontal position. Three way solenoid valves **416** and **420** operate in an identical way, providing communication between the air inlet lines **414** and **418** and the respective air cylinders **280** and **282** when in the open position and actuating respective tipper tips **284** and **286**. They also open communication between the air cylinders **280** and **282** and the atmosphere in the vent position, allowing the tipper tips to return to their elevated position.

Branch line **422** leads from line **408** to the reagent dispenser three way control solenoid valve **424**. When energized to an "open" position, solenoid valve **424** permits pressurized air to pass from line **422** to air cylinder input line **300**, causing rod **302** and foot **306** (FIG. 15) to press the reagent dispenser bottle **12** downward, emitting a precise volume of reagent liquid. When solenoid valve **424** is in the vent position, the air cylinder **18** and the reagent bottle **12** return to their resting positions.

Branch line **426** leads from line **403** to branched lines **428** and **430**. Branch line **428** leads to pressure regulator **38**, providing an output pressure of 10 psi in output line **431**. Three way solenoid valve **432**, when in the open position, provides communication between air input line **431** to the evaporation inhibitor liquid reservoir container **434** through lines **436** and **438**. It also delivers pressurized air to the rinse liquid supply container **44** through line **440**, rinse solution reservoir **441** and supply conduit **443**. When solenoid valve is opened to atmosphere (vent position), air in line **436** and in containers **44** and **434** is bled or vented to the atmosphere. This permits removal, opening or replacement of reservoir container **434**, or opening or removal of supply container **441**. The pressured air in containers **434** and **441** forces liquid through respective output conduits **442** and **443**.

Conduit **442** leads to two way solenoid valve **446**, which has an outlet conduit **448** leading to the evaporation inhibitor application block **212** and associated nozzles. When the solenoid **446** is opened, evaporation inhibitor liquid is emitted from nozzles **294** (FIGS. 14 and 15) onto the surface of the respective slide **234**.

Conduit **444** delivers pressurized rinse liquid from heated rinse liquid container **44** to branch conduits **450**, **452** and

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454 leading to conventional rinse liquid two way solenoid valves **460**, **462** and **464**. When the solenoid valves **460**, **462** and **464** are opened, pressurized rinse liquid is delivered to the respective rinse blocks **200**, **202** and **204** through supply conduits **260**, **268** and **276**. The pressurized rinse liquid is emitted by the rinse blocks onto the slides positioned in the respective station (FIG. 13).

Branch line **430** leads to pressure regulator **64**, providing an output pressure of 15 psi in output conduit **466** leading to vortex mixer air control two way solenoid valve **468**. When in the open position solenoid valve **468** delivers pressurized air to output conduit **470** connected thereto. Conduit **470** leads to branch lines **472** and **474** leading to vortex mixing blocks **222** and **224**. The pressurized air is emitted by nozzles **351** and **355** (FIG. 17), stirring the reagent layer on the respective slides **234**.

FIG. 23 is a schematic drawing of the 120 volt AC power distribution in the apparatus of this invention. The power circuit to power line filter **500** includes a main fuse **504** and main power switch **506**. 120 Volt AC power to the air compressor **80** is provided by line **511** from the line fuse **510** in the I/O board **508**. 120 Volt AC power to the air compressor cooling fan **514** is provided by line **513** from line fuse **512** in the I/O board **508**. 120 Volt AC power to the electronics cooling fan **518** is provided by line **517** from line fuse **516** in the I/O board **508**. 120 Volt AC power to the 24 volt DC power supply is provided by line **521** from line fuse **520** in the I/O board **508**. 120 Volt AC power to the 5 volt/12 volt DC power supply **78** is provided by line **524** from line fuse **522** in the I/O board **508**. 120 Volt AC power to the computer card rack **529** is provided by line **528** from line fuse **526** in the I/O board **508**. 120 Volt AC power to slide heater fan relay **533** is provided by line **532** from line fuse **530** in the I/O board **508**. 120 Volt AC power to the slide heater relays **537** is provided by line **536** from fuse **534** in the I/O board **508**. 120 Volt AC power to the rinse fluid heater relay **541** is provided by line **540** from fuse **538**.

FIG. 24 is a schematic drawing of the DC power distribution in the apparatus of this invention. 12 Volt DC logic power for printer **550** is provided by line **552** from the power supply **78**. Similarly, 12 volt DC power for low slide temperature controller **68** is provided by line **554**, 12 volt power for high slide temperature controller **70** is provided by line **556**, and 12 volt power for rinse fluid temperature controller **66** is provided by line **558**. 5 Volt DC laser power for the slide bar code reader **231** is provided by line **560** from the power supply **78**, and 5 volt power for the laser of reagent bar code reader **346** is provided by line **562**. 5 Volt DC power to the liquid crystal display **34** is provided by line **564**.

24 Volt DC power is provided to the upper motor controller **566** for the stepper motor **14** by line **568**. 24 Volt DC power for the lower motor controller **570** for the stepper motor **48** is provided from power supply **76** by line **572**.

The conventional card rack **529** has a separate 5 volt/12 volt power supply **576**. 5 Volt DC logic power and 12 volt DC motor power is provided to the floppy disc drive by lines **574**.

FIG. 25 is a schematic drawing of a first portion of the computer digital I/O system in the apparatus of this invention. The control system uses a series of standard optical relays, each of which are connected to close the line to ground in the power circuit for the respective component. The optical relays provide isolation.

Communication between the optical relays and the computer digital I/O board **580** is provided by lines **582**. The two

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way solenoid valves **460**, **462** and **464** controlling the rinse liquid flow from heated rinse supply **44** to the respective rinse blocks **200**, **202** and **204** are energized to an open position and de-energized to a closed position by output signals from the computer digital I/O board **580** to the optical relays **584**, **586** and **588**. The two way solenoid valve **446** controlling the flow of evaporation control liquid from container **434** to the nozzle block **212** is energized to an open position or de-energized to a closed position by output signals from board **580** to optical relay **590**.

The three way solenoid valves **412**, **416** and **420** controlling air flow to the respective tipper air cylinders **278**, **280** and **282** are energized to an open position (causing air flow) or de-energized to a closed position (venting cylinder air to the atmosphere) by output signals from computer I/O board **580** to respective optical relays **592**, **594** and **596**. The three way solenoid valve **424** controlling air flow to the micro delivery reagent dispenser control cylinder **300** is energized to an open position (causing air flow and reagent delivery) or de-energized to a closed position (venting cylinder air to the atmosphere) by output signals from computer I/O board **580** to respective optical relay **598**. The two way solenoid valve **468** controlling air flow to the vortex air mixer blocks **220**, **222** and **224** (FIG. 17) is energized to an open position (causing air flow to the mixer blocks) or de-energized to a closed position by output signals from computer I/O board **580** to respective optical relay **600**.

The sound alarm **602** is activated to produce sound by an output signal from the computer I/O board **580** to optical relay **604**. The sound alarm **602** can be activated to sound a 'beep' by keyboard key operation, by a longer 'beep' or double 'beep' at the completion of a run, and a sustained sound during a system malfunction, for example. The three way solenoid valve **432** controlling air flow to the rinse liquid and evaporation control liquid supply containers **44** and **434** (FIG. 22) is energized to an open position (causing air flow and pressurization of the supply containers) or de-energized to a closed position (venting cylinder air from the containers to the atmosphere) by output signals from computer I/O board **580** to respective optical relay **606**.

The slide heat fan **56** speed is operated by pulse width modulation, that is, power pulses from the power relay **608**. The fan **56** is energized by an output signal to the power relay **608** from optical relay **610**. The timed signal to the optical relay **610** is received from the computer I/O board **580**. The pulse width and speed of the fan **56** is adjusted in response to heating requests from the high temperature slide controller **632** to increase the volume of heating air delivered to the air distribution manifold **30**.

The slide heater system control supplies separately controlled power to each of the resistance heating elements **331** and **332**. Low temperature heating element **332** is energized by power relay **612** upon a signal from the low slide temperature controller **614**. Thermistor **347** provides temperature information to the controller **614**. During the operation of the apparatus at the lower temperatures required for the immunohistological processes, the power to the heating element **332** is turned on when operating heat is required, in response to a low temperature signal from the low temperature controller **614**. It is turned off when the operating temperature is restored. The controller **614** also detects when the slide door switch **616** is closed. If the cabinet slide door is open, energy supply to the heating element **331** and **332** is interrupted. The heating cycle is initiated by a request for heat passed to the computer I/O board **580** through line **624** to the optical relay **622**. The computer then responds with a heating power select heat signal received by controller **614**

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through line **620** from optical relay **618** in response to an output signal from the computer I/O board **580**. A status signal for the slide door switch is received by the computer I/O board through line **628** and optical relay **626**.

The high temperature heating element **331** is energized by power relay **630** upon a signal from the high slide temperature controller **632**, in response to a power command signal through optical relay **634** and line **636** from the computer digital I/O board **580**. During the operation of the apparatus at the lower temperatures required for the immunohistological processes, the power to the heating element **331** is turned on only during an initial warm-up cycle. During the warm-up cycle, heat energy is requested from the I/O board **580** through line **638** and optical relay **640**.

When the apparatus is operated at the higher temperatures required for in situ hybridization, the heating elements are energized in a different control sequence by the controllers **614** and **632**. As with the low temperature operation, both heating elements **331** and **332** are energized during the warm-up cycle. However, in the high temperature operating mode, the low temperature heating element **332** is continuously energized, and energy is supplied intermittently to the heating element **331**. In the high temperature mode, therefore, the optical relay **634** receives a power command signal from the I/O output board **580** when the high temperature controller **632** signals that more heat is required. In addition to the heater controls described above, an additional thermostat is provided in the heater circuit which turns the heater off if the heater temperature reaches 160° C., for example if the fan **56** fails.

The rinse liquid heating system resistance heater **390** (FIG. 19) is energized through power relay **642** upon a signal from rinse fluid controller **644**. Thermistor **391** monitors the rinse fluid temperature, and the controller **644** provides a signal indicating whether or not further heat energy is required. A heat request signal for heating liquid is received by the computer I/O board through line **646** and optical relay **648**. The computer responds with a heat select signal from the I/O board **680** through relay **650** and line **652**.

FIG. 26 is a schematic drawing of a second portion of the computer digital I/O system in the apparatus of this invention. The computer digital I/O board **580** receives a signal indicating closure of the air pressure switch **402** (FIG. 22) through line **670** and optical relay **672**. The computer digital I/O board **580** receives a home signal from the reagent carousel metal proximity home sensor through line **676** and optical relay **674** when the metal block **803** and the reagent carousel **10** are in the home position. The computer digital I/O board **580** receives a home signal from the slide support metal proximity home sensor **610** through line **680** and optical relay **678** when the metal block **229** and the slide support carousel **24** are in the home position.

The reagent carousel stepper motor **14** is operated by reagent carousel stepper motor controller **690** in response to commands received from the computer digital I/O board **580**. Command signals for steps (motor operation) are received through line **692**, and command signals for the direction of operation are received through line **694**. The stepper motor has a high and low torque operating mode, the low torque mode being effected by switching a resistor into the control circuit. The high torque mode is used to move the motor through the number of steps required to place a selected reagent bottle in the reagent delivery station. The low torque mode is used as a brake to hold the reagent bottle carousel in a position. The low or high torque command signal is received by the reagent carousel stepper motor controller **690** through line **698** and optical relay **696**.

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The slide support carousel stepper motor **48** is operated by slide support carousel stepper motor controller **700** in response to commands received from the computer digital I/O board **580**. Command signals for steps (motor operation) are received through line **702**, and command signals for the direction of operation are received through line **704**. This stepper motor also has a high and low torque operating mode, activated in the same way and having the same functions as the reagent carousel stepper motor operating modes. The high torque mode is used to move the motor through the number of steps required to place a selected slide in a selected treatment zone. The low or high torque command signal is received by the slide support carousel stepper motor controller **700** through line **708** and optical relay **706**. When the door switch **616** shows an open door status, the step command signals to the stepper motors **14** and **48** are prevented. If the door switch **616** is opened during a biological processing run, any incomplete stepper motor sequence is permitted to reach completion before further step command signals are blocked.

The keyboard **710** is a conventional pressure sensitive keyboard. The switches **720–726**, **730–736**, **740–746** and **750–756** are closed by manual pressure applied to the surface of an impermeable flexible plastic layer over the switches. The switches are isolated and protected under the plastic layer and are not fouled by moisture or debris from the laboratory or operator.

In operation input lines **711**, **712**, **714** and **716** are each sequentially energized for a brief period by the computer digital I/O board **580**, and the lines **718**, **728**, **738** and **740** are each sequentially polled during this brief period. If line **718** polls positive while line **716** is energized, closure of switch **720** is indicated. In a similar manner, closure of switch **722** is indicated by a positive poll of line **718** when line **714** is energized, closure of switch **724** is indicated by a positive poll of line **718** when line **712** is energized, closure of switch **726** is indicated by a positive poll of line **718** when line **711** is energized, and the like.

FIG. **27** is schematic drawing of the computer serial and floppy disk I/O system in the apparatus of this invention. The computer RS-232 I/O port **770** sends polling signal to the slide barcode reader **231** and receives signals indicating bar code information read through line **772**. Similarly, the computer RS-232 I/O port **770** sends polling signal to the reagent carousel barcode reader **346** and receives signals indicating barcode information read through line **774**. Signals to the liquid crystal display **34** are sent through line **776** from the RS-232 I/O port **770**. The computer RS-232 I/O port **770** receives an availability polling signal from the printer **550** and sends digital data to printer **550** through line **778**.

Immunohistological methods for which the apparatus of this invention are particularly suitable are described in concurrently filed, commonly assigned patent application Ser. No. 07/488,601, filed Mar. 2, 1990, now abandoned, the entire contents of which are hereby incorporated by reference. A typical immunohistological method, as carried out with the apparatus of this invention includes the following steps.

- 1) Preparing the slides, including applying a bar code to the slide indicating the immunohistological process to be used with the sample, and manually rinsing and applying evaporation inhibiting liquid to the tissue sample surface before placement in the apparatus to prevent dehydration of the sample.
- 2) Inserting a batch of slides in the apparatus, mounting each slide in a slide support.
- 3) Closing the apparatus and beginning the treatment processing. The apparatus heating system is in the warm-up mode until the heating air temperature reaches the desired level.

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- 4) A slide is rinsed in the first rinse station (FIGS. **11–14**) in seven rinse cycles. Each cycle includes applying a 500 μ L pulse of rinse liquid followed by tipping the slide support to effect draining. This sequence can be repeated for seven rinse cycles as the slide is moved to and pauses in each of the second and third rinse stations, for a total of twenty-one rinse cycles, for example. The slide then is treated in a seven second stay in the evaporation inhibitor and reagent solution application station (FIGS. **11**, **14** and **15**). An initial quantity of 500 μ L of an evaporation inhibiting liquid such as dodecane is applied to the slide surface. Then 200 μ L of reagent solution is applied to the slide.

As each slide poises in the reagent application zone, the appropriate reagent container is moved by the reagent carousel to the reagent application station, and a metered volume of reagent is applied to the slide. In being applied to the slide, the reagent liquid is applied to the uppermost surface (the evaporation liquid layer). It then passes through the evaporation inhibiting liquid layer to the underlying aqueous layer, a procedure which would not be possible with a conventional solid glass coverslip.

- 6) The slide is then passed to each of the vortex mixing stations (FIGS. **11**, **14**, **16** and **17**). Here vortex jets stir the reagent on the slide surface under the file of evaporation inhibiting liquid. This procedure would not be possible with a conventional solid glass coverslip.
- 7) The slide is then carried by the carousel, pausing as each slide support is sequenced through the same steps, until it returns to the initial rinse station, where the cycle is repeated. The reaction between the reagent and the tissue sample continues during this period, and slides in each of the following slide supports is subjected to the same sequence of rinse, application of evaporation inhibitor, application of reagent, stirring, and incubation.

- 8) In a typical immunohistological process using a four phase process with a peroxidase enzyme antibody label, a sequence total of five different reagents are applied as the tissue sample is passed five times through the reagent application zone. In such a process, the first reagent is a hydrogen peroxide solution required to eliminate endogenous peroxidase activity in the tissue sample. The second reagent is a primary antibody which binds selectively with an specific epitope for which the sample is being tested. The third reagent is a biotin labeled secondary antibody which binds preferentially with the primary antibody remaining on the sample following the preceding incubation and rinsing. The fourth reagent is avidin labeled with an enzyme such as a peroxidase enzyme, the avidin binding with the biotin label remaining on the sample following the preceding incubation and rinsing. The fifth reagent is a substrate solution which is converted by the peroxidase enzyme to form a detectable label such as a fluorophore or chromophore at the site of any primary antibody binding with the sample.

- 9) Following the conclusion of the substrate solution treatment and incubation, the slide typically is removed from the carousel, coverslipped with a glass coverslip and examined to determine the extent of primary antibody binding with the tissue sample.

FIG. **28** illustrates an alternative embodiment of the intermediate section **4**, including the slide support carousel **24** and the associated slide treatment stations, which dispenses with the tipper rinse method described above and employs an alternative rinsing arrangement, using stationary slide supports, as will be more fully described hereinafter. The carousel **24** is rotated, for example, in a clockwise manner, as indicated by the arrow shown in FIG. **28**, so that

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each slide support **26A** and associated slide **234** is positioned in the rinse zone A, evaporator inhibitor and reagent application zone B, and agitation zone C for successive treatment and incubation as previously described above.

In the embodiment depicted by FIG. **28**, an alternative embodiment of the slide support **26A** is provided which does not pivot, but rather is fixedly supported in a predetermined position on the carousel **24** by screws or the like and structured so that the associated slide **234** is held substantially horizontally as best seen in FIGS. **29A–29B**. Referring to FIGS. **29A–29B**, the slide support **26A** has a distal end **103A**, which is juxtaposed to the center of the carousel **24**, and a proximal end **104**, which is positioned adjacent to an outer circumference of the carousel **24**.

The support **26A** comprises a support plate **102A** having a raised terminal guide end platform **106**, adjacent the proximal end **104A** and a support post **107A**, adjacent the distal end **103A**. The platform **106A** and the post **107A** cooperate to support the slide **234** in a substantially horizontal position at a predetermined vertical distance with respect to raised terminal guide tabs **108A** and **109A** between which the slide **234** is positioned.

As best seen in FIG. **29B**, the tabs **108A**, **109A** are provided with a vertical length such that the upper surface of the slide **234** is positioned above the upper ends of the guide tabs **108A**, **109A** while the respective lateral edges **111A**, **113A** of the tabs **108A**, **109A** engage the lateral sides of the slide **234**, i.e., the tabs **108A** and **109A** do not extend as far as the upper surface of the slide **234** to prevent wicking-off of any liquid on the upper surface of the slide **234** by the tabs **108A** and **109A**. The lateral edges **111A**, **113A** cooperate with the a guide edge **115A** at the platform **106A** to orient the slide **234** at a predetermined position with respect to the slide support **26A**, and thus the carousel **24**, for treatment at the various treatment stations to be describe hereinafter.

A clamping arrangement, generally indicated at **118A**, positioned at the proximal end **104A**, clamps the slide **234** to the slide support **26A**. The clamping arrangement comprises a pair of supports **119A** between which a slide engaging member **120A** is pivotally supported. Spring **121A** biases the slide engaging member **120A** to firmly hold the slide **234** against the platform **106A** and post **107A**. The slide support **26A** permits easy loading and unloading of the slide **234**, firmly holds the slide **234** in place, does not interfere with the operation of the bar code reader and prevents or minimizes the wicking, i.e., surface tension, from draining liquids off the slide **234**.

An alternative embodiment of the rinsing arrangement forming the rinse zone A is employed in the embodiment depicted by FIG. **28** which replaces the rinse blocks, and arrangement thereof, used with the tipper rinse method previously described with respect to FIG. **14**. Referring to FIG. **28**, the rinse zone A employs a first rinse block **200A**, having a single wash block nozzle, as best seen in FIGS. **30A–30B**, and a second rinse block **202A**, having a dual wash block nozzle, as best seen in FIG. **31**.

The first wash block **200A** is preferably positioned at the beginning of the rinse zone A and the second wash block **202A** is preferably positioned at the end of the rinse zone A so that the first and second wash blocks are spaced from one another. The first wash block **200A** pulses streams of rinse liquid onto a slide upon entering the rinse zone A and due to the meniscus effect of the rinse liquid at the edges of the slide, builds up a layer of rinse liquid which remains on the slide. After a predetermined waiting period, set by the time it takes for the slide carousel to transport a slide between the first and second wash blocks **200A**, **202A**, the second wash

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block **202A** uses pulsed streams of rinse liquid, alternately directed at one and then the other of the longitudinal edges of the slides, to knock or sweep the previously deposited layer of rinse liquid off of the slide.

The rinsing arrangement depicted in FIG. **28** rinses or washes the upper surface of the slides with streams or jets of pulsed rinsing liquid, for example, water, so that a low volume of rinsing liquid is used to provide a high degree of rinsing. Because the rinsing of the slides is a key limit to the sensitivity of the assays as background or noise is directly related to rinsing and sensitivity is the signal to noise ratio, the wash blocks **200A**, **202A** precede the application of the reagent and are a preferred feature of this embodiment of the invention.

Referring to FIG. **30A**, the first wash block **200A** comprises a single wash block nozzle **201A** having a plurality of nozzle outlet openings **203A**, for example 10 or so openings, which each provide a pulsed stream of rinse liquid **204A** which impacts the rinse liquid impact zone **236** of the slide **234** as previously described. Due to the meniscus effect of the rinse liquid at the longitudinal edges **234P** and lateral edge **234L** of the slide **234**, a layer of rinse liquid **213A** is built up on the slide **234** as a result of the repeated pulsing of streams of rinse liquid during the operation of the first wash block **200A**.

As best seen in FIG. **30B**, a nozzle axis **240A** of the nozzles of block **200A** forms an angle θ with the horizontal, this angle being between 15 and 35 degrees, preferably substantially 25 degrees.

FIG. **31** illustrates the second wash block **202A** which employs a dual wash block nozzle **205A** comprising a lower set of nozzle outlet openings **206A** and an upper set of nozzle outlet openings **207A** which respectively direct streams of pulsed rinse liquid towards one or the other of the longitudinal edges **234P** of the slide **234**.

As with the first wash block **200A**, the streams of pulsed rinsing liquid, from each of the lower and upper sets of nozzle outlet openings **206A** and **207A**, preferably impact the slide **234** at the rinse liquid impact zone **236** which is upstream on the slide **234** from the tissue sample (not shown) positioned thereon. This feature of the first and second wash blocks **200A** and **202A** is important due to the fragile nature of the tissue sample positioned on the slide **234**. By directing the streams of pulsed rinsing liquid at the impact zone **236** of the slide **234**, the rinse liquid is provided with laminar flow by the time the rinse liquid reaches the tissue sample. As a result, undue damage to the fragile tissue sample is prevented.

The upper set of nozzle outlet openings **207A** is constructed so that the associated streams of rinse liquid are off-set at an angle from the longitudinal center line of the slide **234** so that the pulsed streams of rinse liquid are directed toward one of the longitudinal edges **234P** of the slide **234**. The lower set of nozzle openings **206A** is constructed so that the associated streams of rinsing liquid are also off-set at an angle from the longitudinal center line of the slide **234** so that the pulsed streams of rinse liquid are directed toward the other one of the longitudinal edges **234P** of the slide **234**. As a result of this arrangement, pulsed streams of rinse liquid are alternately and repeatedly directed to one and then the other of the longitudinal edges **234P** of the slide **234** as will be more fully described hereinafter.

Preferably, separate plumbing and valving are provided for each of the lower and upper sets of nozzle outlet openings **206A** and **207A** of the dual wash block nozzle **205A** to permit independent operation thereof. In operation,

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wash block **202A** directs streams of pulsed rinsing liquid, for example from the lower set of nozzle openings **206A**, toward a single longitudinal edge **234P** of the slide **234** and after completion then directs streams of pulsed rinse liquid, for example from the upper set of nozzle opening **207A**, to the other longitudinal edge **234P** of the slide **234**. This procedure is repeated and has the effect of sweeping or knocking the layer of rinse liquid **213A** off of the slide **234**.

As with the first wash block **200A**, the nozzle axis **240** (not shown) of each of the upper and lower set of nozzle openings **207A**, **206A** forms an angle θ (not shown) with the horizontal of between 15 and 35 degrees, preferably substantially 35 degrees for the upper set of openings **207A** and substantially 25 degrees for the lower set of openings **206A**.

FIG. **32** illustrates an alternative embodiment of a vortex air mixer **220A** which in this case is a single mixer. Each of the single vortex air mixers **220A** is positioned at the inner radius of the slides **234** such that an gas jet or cone **356A** of, for example, air or the like, blows outwardly adjacent one of the longitudinal lateral edges **234P** of the associated slide **234** to effect mixing in a manner similar to that described with respect to FIG. **17**. More specifically, the gas stream **356A** impacts the surface of the evaporation liquid surface layer **360** and moves the underlying reagent solution in a circular path on the tissue section.

Each vortex mixer **220A** has a nozzle channel **350A**, including a nozzle orifice **351A**, which is supplied with pressurized air via a supply channel **358A**, the nozzle channel **350A** preferably intersecting the supply channel at a lower portion thereof. Pressurized air is supplied to the supply channel **358A** from a air supply conduit **352A** (arrows indicating the flow of air to and from the mixer **220A**) connected to a pressurized air source (not shown). Each of the vortex mixers **220A** can be supplied with pressurized air via a common supply conduit **352A** which connects and supplies each of the supply channels **358A** of the plurality of mixers **220A** illustrated in FIG. **28**.

As best seen in FIG. **28**, there are, for example twelve, single vortex mixers **220A** on the inner radius of the slides **234**. The nozzle orifice **351A** of each of the mixers **220A** is preferable positioned so that the center of the gas jet or cone **356A** is approximately 2 mm above the surface of the slide **234** and 4 mm in from the adjacent edge **234X** of the slide **234** as best seen in FIG. **32**.

A first mixer **220A** is preferably positioned at station **S2** adjacent the reagent drop point station **S1** and a second mixer **220A** is positioned at station **S3**, the mixers **220A** at stations **S2** and **S3** directing the stream of air **356A** to opposite longitudinal edges **234P** of an associated slide **234** so that mixing is enhanced as described below.

The exact positioning of the remaining mixers **220A** is not critical, these mixers **220A** being positioned to provide a semi-continuous mixing. Additionally, each mixer **220A** is spaced so that they alternate in blowing the right side and then the left side of the slide **234**. That is, the even mixers blow up the right side of each slide **234** passing by and the odd mixers blow up the left side or vice versa. This enhances kinetic mixing, provides uniform coverage and averages out any possible temperature differences across each of the slides **234**. These features lead to more rapid and reproducible staining than can be obtained manually.

Additionally, the intermediate section **4** of the embodiment of FIG. **28** is provided with a bar code cleaner, generally indicated at **233A**, for cleaning drops of liquid off of the bar codes **233** (FIG. **32**) provided for each of the slides **234** for identification purpose as previously described. It should be noted that the bar code cleaner **233A** is equally

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applicable to the previously described embodiment of the invention employing the tipper rinse method describe above. The bar code cleaner **233A** is positioned, for example, downstream from the reagent drop point station **S1** just beyond the first vortex agitation zone **C** as best seen in FIG. **28** and upstream and adjacent to the bar code reader position (not shown).

The bar code cleaner **223A** is illustrated in detail in FIGS. **33A–33B** and comprises a bar code nozzle **333A** supplied with compressed air or the like via a supply channel **334A** which is connected to a compressed air source (not shown) by supply conduit **335A**. The bar code nozzle **333A** is supported above the slide carousel **24** by support **336A**, as best seen in FIG. **33B**, and affixed to the stationary support plate **22** of the intermediate section **4**. The nozzle **333A** emits a stream or cone of air **337A** which blows across the bar code **233** of an adjacent slide **234** attached to the associated slide support **26A**. The stream of air **337A** blows drops of liquid off of the bar code **233** which otherwise interfere with the reading of the bar codes by the bar code reader.

As best seen in FIG. **33A**, the nozzle axis **338A** of the bar code nozzle **333A** forms an angle of about 45 degrees with the horizontal. Additionally, the stream of air **337A** preferably strikes the bar code **233A** in the area of the side of the bar code **233A** closest to nozzle **333A**.

Since the embodiment of the intermediate section **4** described with reference to FIG. **28** does not employ the tipper rinse method, any rinse liquid remaining on the slide after operation of the second wash block **202A** is drained from the upper surface of the slides **234** by a jet drain **148A** which is illustrated schematically by FIG. **34**. The preferred position of the jet drain **148A** is at the last rinse station of the rinse zone **A** just prior to the reagent drop point station **S1** as best seen in FIG. **28**.

The jet drain **148A** directs a fluid stream **150A** of, for example air, at substantially a 45 degree angle to the longitudinal axis of an associated slide **234** and across one corner of the distal end **104A** of the associated slide **234**. The action of the fluid stream **150A** acts to blow, aspirate or siphon the buffer remaining after the rinsing performed at the rinse zone **A** as described above.

Except for the differences noted above the embodiment so described with respect to FIG. **28** is the same as the apparatus described above in connection with the tipper rinse method and is capable of operating and performing the immunohistological methods as previously described.

What is claimed is:

1. A method of dispensing reagents onto a slide, the method comprising the steps of:

- providing at least one reagent container;
- providing at least one slide on a slide support;
 - automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container;
- automatically determining whether reagent in the reagent container should be dispensed onto the slide; and
- dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide wherein the step of automatically determining whether reagent in the reagent container should be dispensed onto the slide includes identifying barcode information from the slide.

2. The method of claim 1, further comprising the steps of: determining a position of the slide; and

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correlating the position of the slide with the information identifying the slide.

3. The method of claim 1, wherein the step of identifying information from the slide includes reading the slide barcode.

4. The method of claim 3, further comprising the steps of: determining a position of the slide; and correlating the position of the slide with the slide barcode.

5. The method of claim 4, wherein the step of determining a position of the slide includes determining a position of the slide relative to a slide carousel home position.

6. The method of claim 1, wherein the information associated with the reagent container includes a reagent barcode.

7. The method of claim 6, wherein the reagent barcode is placed on the reagent container.

8. The method of claim 1, wherein the step of automatically identifying the reagent container using a computer includes the steps of:

providing a bar code reader;

reading a reagent bar code associated with the reagent container using the bar code reader thereby acquiring reagent information; and

sending the reagent information to the computer.

9. The method of claim 8, further comprising the steps of: determining position information for the reagent container; and

sending the position information to the computer.

10. The method of claim 9, wherein a reagent carousel supports the reagent container and wherein the step of determining position information for the reagent container includes homing the reagent carousel and determining an indexed position of a motor drive for the reagent container.

11. The method of claim 8, wherein the reagent bar code identifies the reagent in the reagent container.

12. The method of claim 11, wherein the step of automatically identifying the reagent container using a computer is performed at a beginning of a slide treatment operation.

13. The method of claim 12, wherein the step of automatically identifying the reagent container using a computer further includes correlating a position of the reagent container with the reagent carousel.

14. The method of claim 8, wherein the reagent container is in a reagent carousel and wherein the step of automatically identifying reagent further includes the step of rotating the reagent carousel so that the reagent bar code on the reagent container is read by the bar code reader.

15. The method of claim 1, wherein the step of dispensing the reagent in the reagent container onto the slide includes the step of mechanically actuating the reagent container thereby metering a volume of reagent onto the slide.

16. The method of claim 15, wherein the step of mechanically actuating the reagent container includes activating an actuator to move into positive contact with the reagent container.

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17. A method of dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container;

providing at least one slide on a slide support;

automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container;

automatically determining whether the reagent in the reagent container should be dispensed onto the slide;

moving the reagent container and the slide support relative to one another to position the reagent container over the slide; and

dispensing the reagent in the reagent container onto the slide based on the determination of whether the reagent in the reagent container should be dispensed onto the slide.

18. The method of claim 17, wherein a reagent carousel supports the reagent container and wherein the step of moving the reagent container and the slide support relative to one another includes moving a drive plate which supports the reagent carousel to place the reagent container in a reagent delivery zone.

19. The method of claim 17, wherein the information associated with the reagent container includes a reagent barcode.

20. The method of claim 19, wherein the reagent barcode is placed on the reagent container.

21. The method of claim 17, wherein the step of automatically identifying the reagent container using a computer includes the steps of:

providing a bar code reader;

reading a reagent bar code placed on the reagent container using the bar code reader; and

sending information from the reading of the reagent bar code to the computer.

22. The method of claim 21, wherein the reagent container is supported on a reagent carousel and wherein the step of moving the reagent container and the slide support relative to one another includes moving a drive plate which supports the reagent carousel to place the reagent container in a reagent delivery zone.

23. The method of claim 17, wherein the step of dispensing the reagent in the reagent container onto the slide includes the steps of:

pushing downward on the reagent container; thereby

applying a metered volume of reagent onto the slide.

24. The method of claim 23, wherein the step of pushing downward on the reagent container includes activating actuator to move into positive contact with the reagent container.

* * * * *

EXHIBIT

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US005235167A

United States Patent [19]

Dvorkis et al.

[11] **Patent Number:** 5,235,167[45] **Date of Patent:** Aug. 10, 1993

- [54] **LASER SCANNING SYSTEM AND SCANNING METHOD FOR READING BAR CODES**
- [75] **Inventors:** Paul Dvorkis, Stony Brook; David P. Goren, Ronkonkoma; Glenn S. Spitz, Far Rockaway, all of N.Y.
- [73] **Assignee:** Symbol Technologies, Inc., Bohemia, N.Y.
- [21] **Appl. No.:** 715,267
- [22] **Filed:** Jun. 14, 1991

Related U.S. Application Data

- [63] Continuation-in-part of Ser. No. 506,674, Apr. 9, 1990, abandoned, which is a continuation of Ser. No. 260,692, Oct. 21, 1989, Pat. No. 4,933,538.
- [51] **Int. Cl.⁵** G06K 7/10
- [52] **U.S. Cl.** 235/462; 235/472; 235/467
- [58] **Field of Search** 235/467, 470, 462
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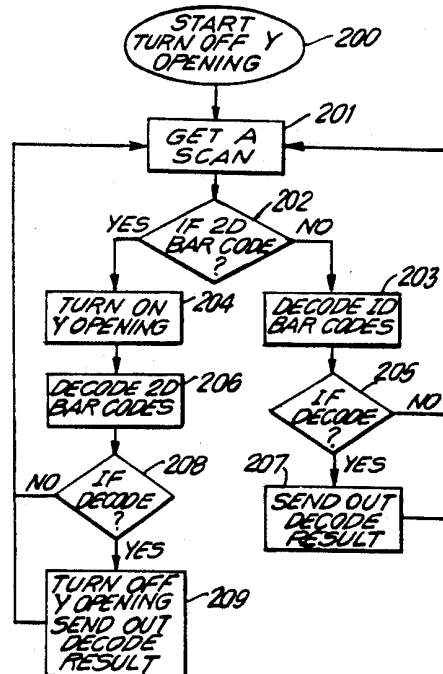
- 0384955 7/1990 European Pat. Off. 235/470

Primary Examiner—John Shepperd
Assistant Examiner—Esther Chin

[57] **ABSTRACT**

A system for reading bar code symbols or the like, including one- or two-dimensional bar code symbols having a scanner for generating a laser beam directed toward a target and producing a narrow first raster scanning pattern that enables the user to manually aim and direct the beam to the location desired by the user and a second raster scanning pattern that increases in height which sweeps an entire symbol to be read, and a detector for receiving reflected light from such symbol to produce electrical signals corresponding to data represented by such symbol.

72 Claims, 9 Drawing Sheets

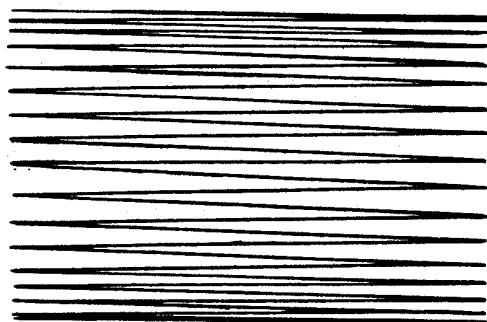
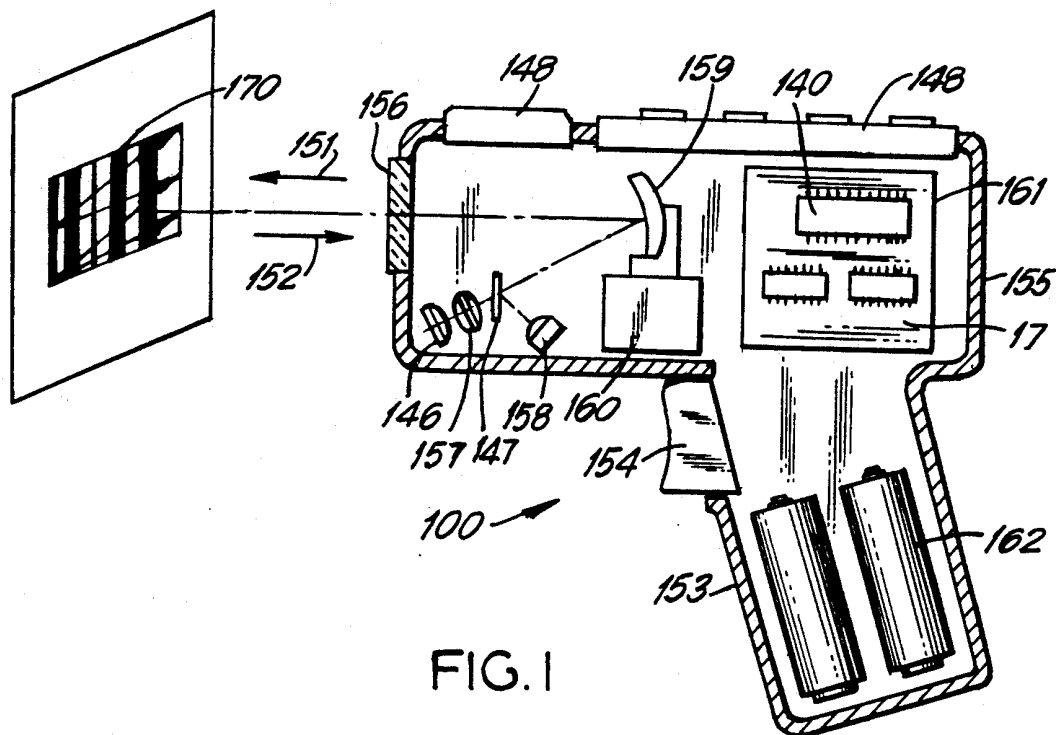


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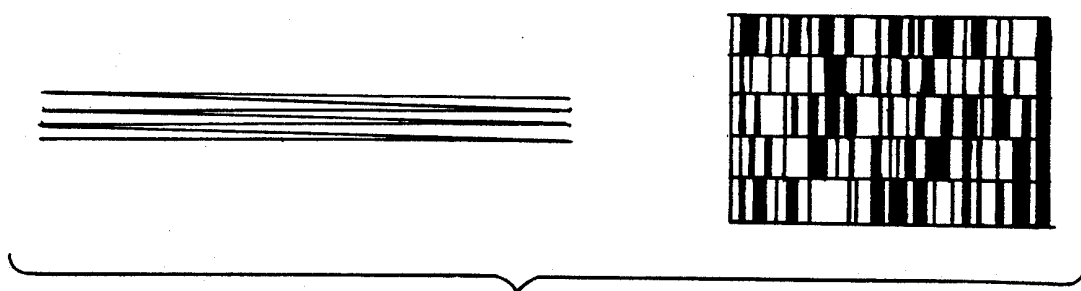


FIG. 3a

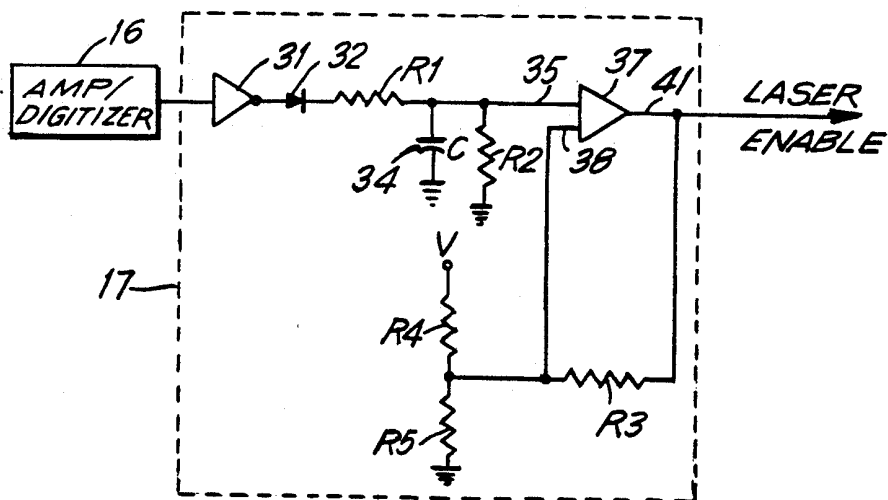


FIG. 6

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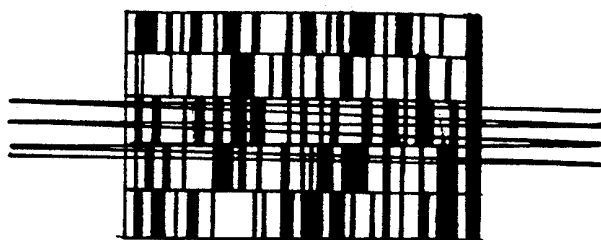


FIG. 3b

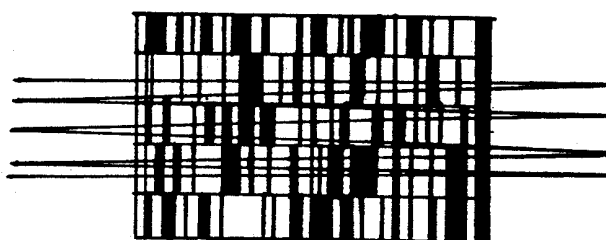


FIG. 3c

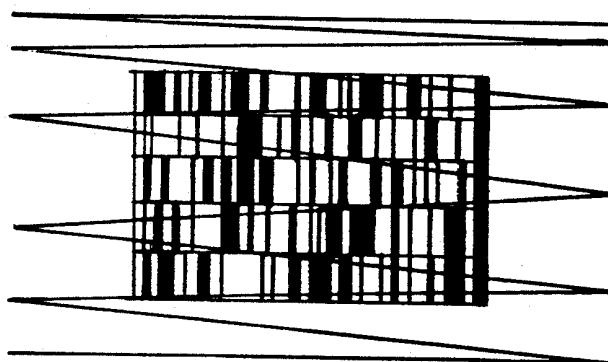


FIG. 3d

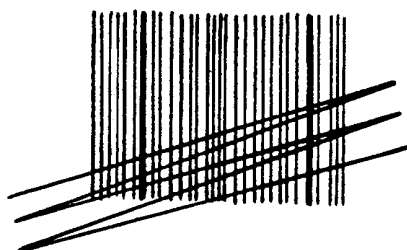


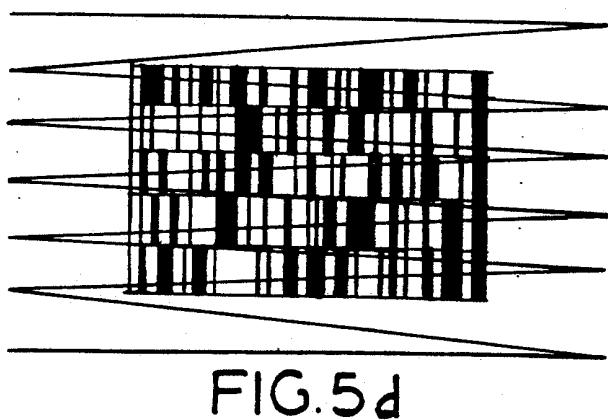
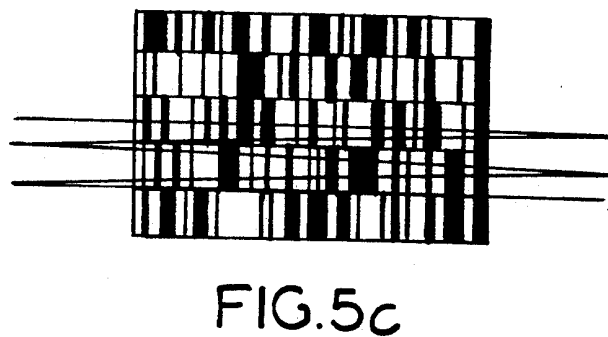
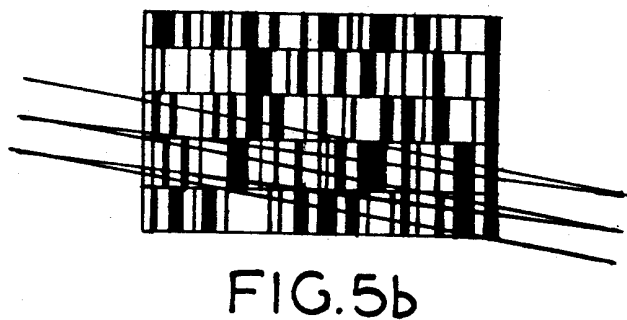
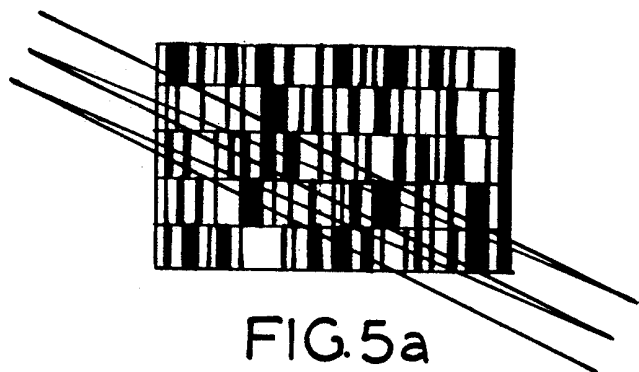
FIG. 4

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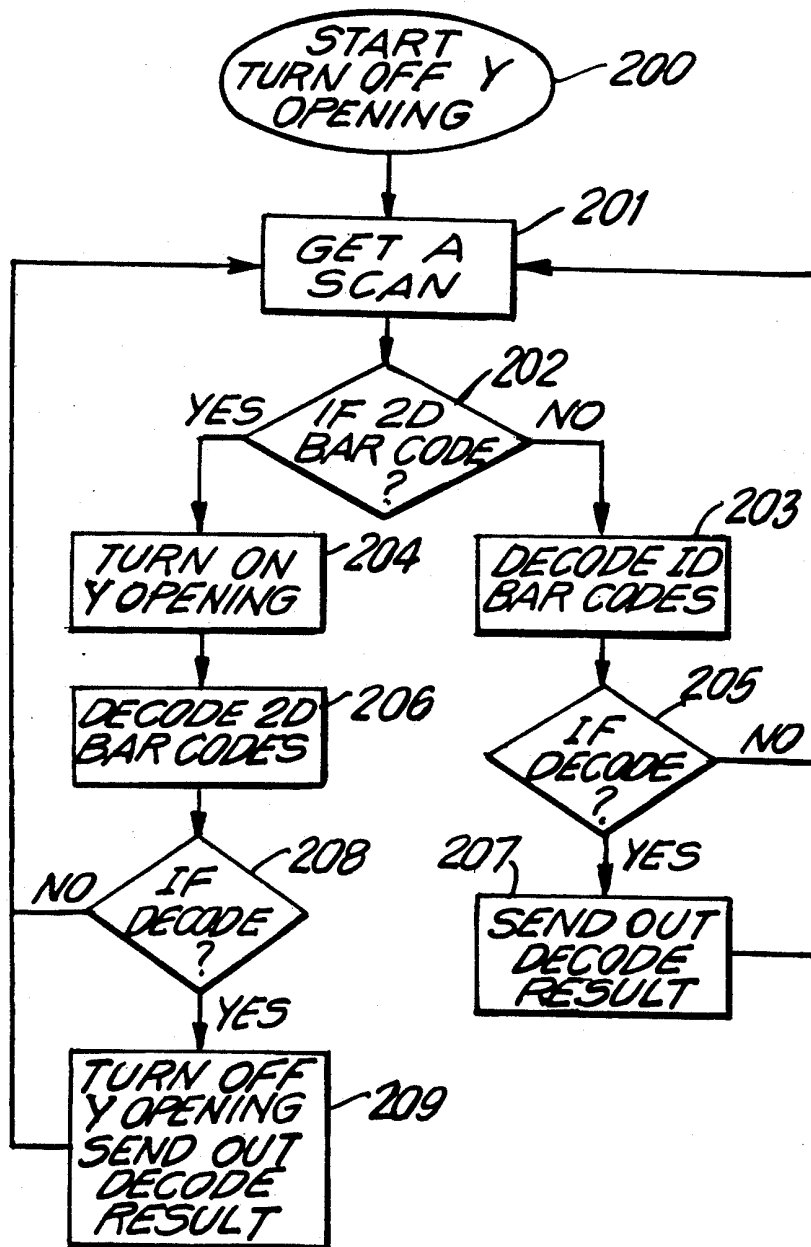


FIG. 7

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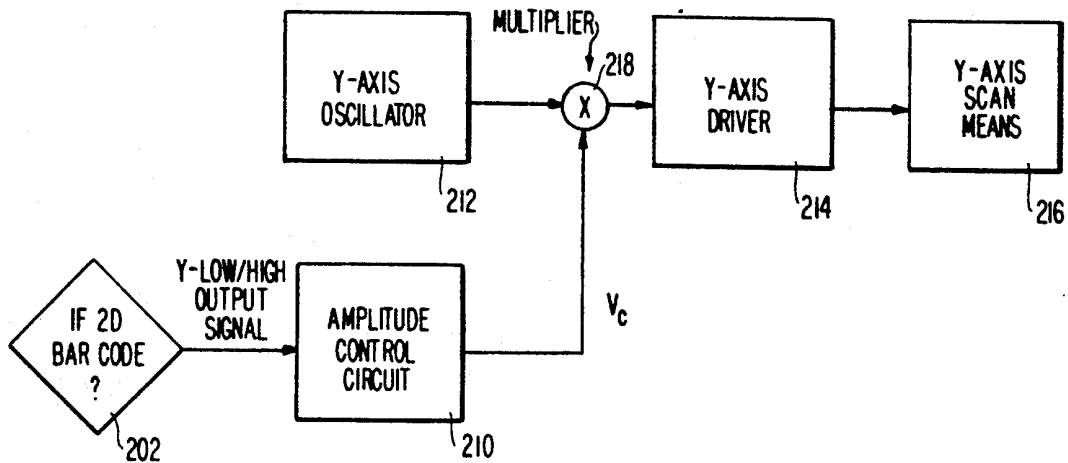


FIG. 8

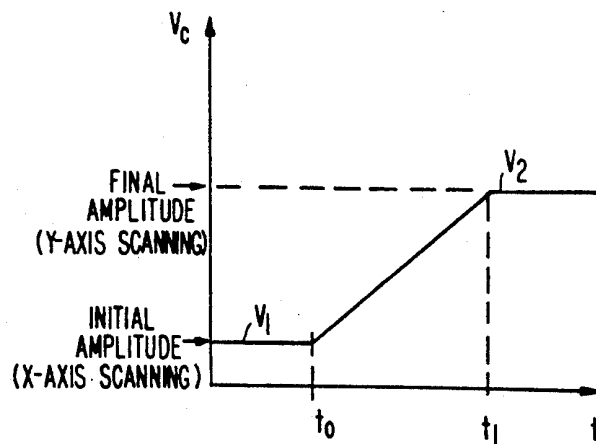


FIG. 9

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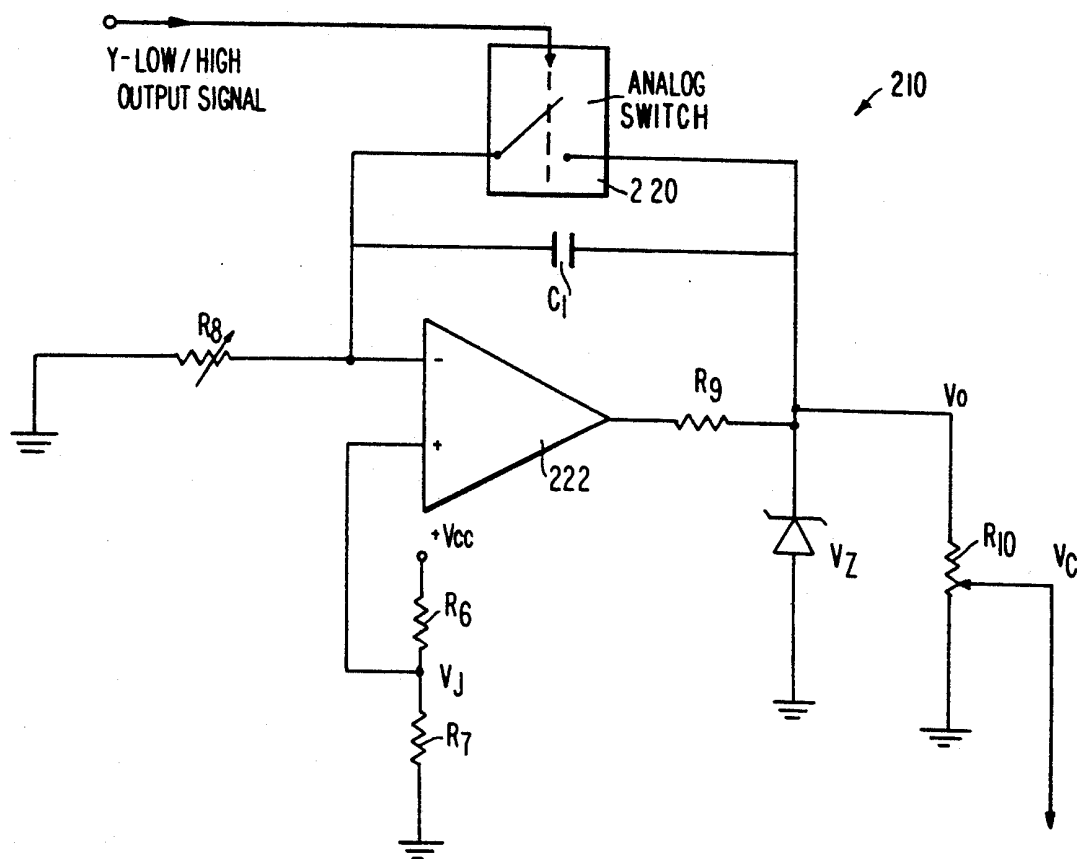


FIG. 10

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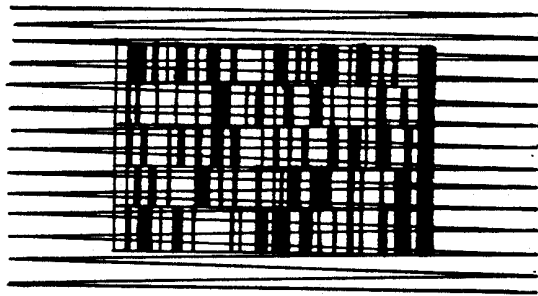


FIG. 11a

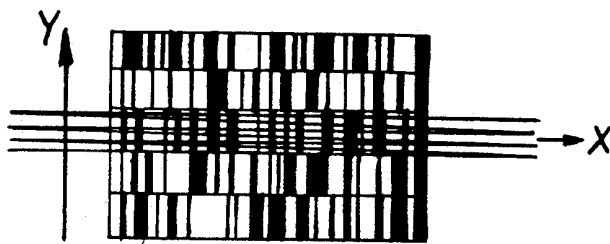


FIG. 11b

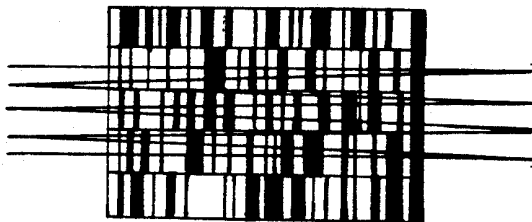


FIG. 11c

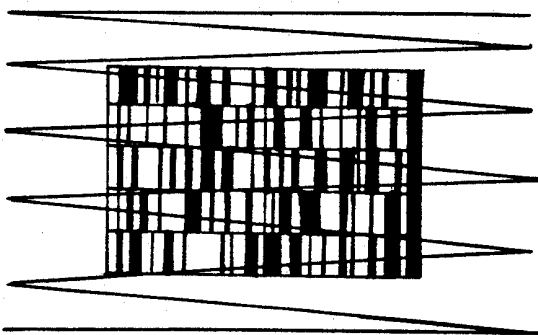


FIG. 11d

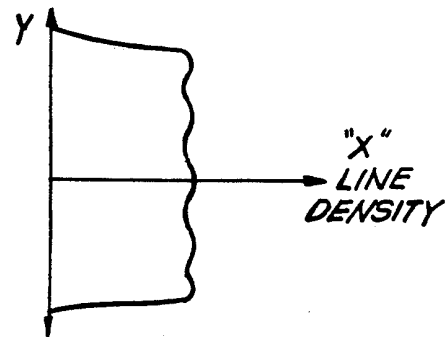


FIG. 12a

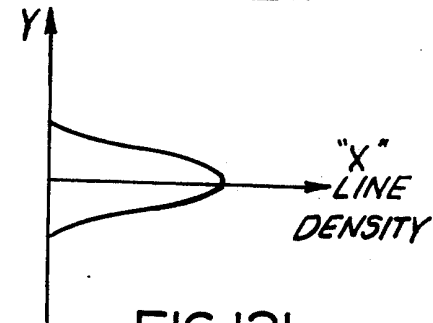


FIG. 12b

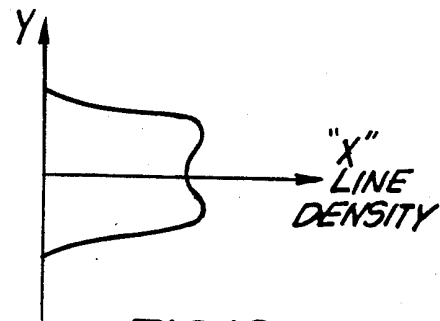


FIG. 12c

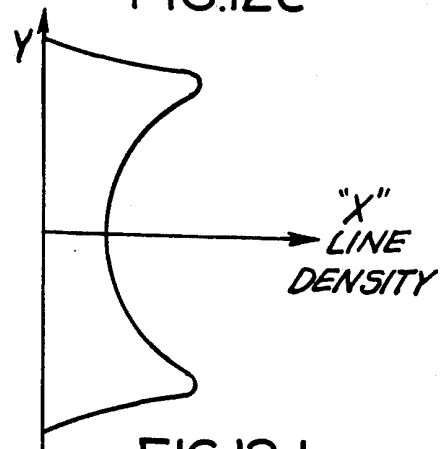


FIG. 12d

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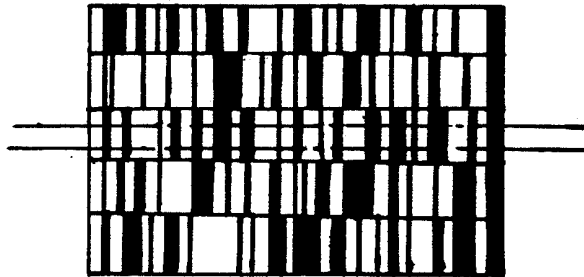


FIG. 13a

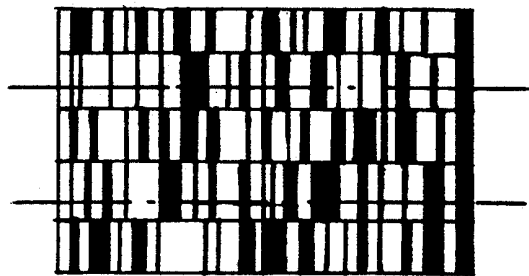


FIG. 13b

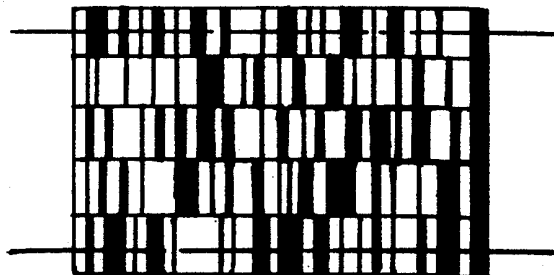


FIG. 13c

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LASER SCANNING SYSTEM AND SCANNING METHOD FOR READING BAR CODES

RELATED CASES

This application is a continuation in part of U.S. patent application Ser. No. 506,674, filed Apr. 9, 1990, abandoned Jan. 6, 1992, which in turn is a continuation of Ser. No. 260,692, filed Oct. 21, 1989, now U.S. Pat. No. 4,933,538. This application is further related to U.S. patent application Ser. No. 317,433, filed Mar. 1, 1989, to U.S. Pat. No. 5,168,149 and U.S. Pat. No. 5,117,098 all of said applications being assigned to Symbol Technologies, Inc.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention generally relates to the design of laser scanning systems for reading bar code symbols or similar indicia and, more particularly, to method of changing the scanning pattern of a raster scan in order to more effectively read two dimensional bar code symbols.

2. Description of the Related Art

Various optical readers and optical scanning systems have been developed heretofore for reading bar code symbols appearing on a label or on the surface of an article. The bar code symbol itself is a coded pattern of indicia comprised of a series of bars of various widths spaced apart from one another to bound spaces of various widths, the bars and spaces having different light-reflecting characteristics. The readers and scanning systems electro-optically transform the graphic indicia into electrical signals, which are decoded into alphanumeric characters that are intended to be descriptive of the article or some characteristic thereof. Such characters are typically represented in digital form and utilized as an input to a data processing system for applications in point-of-sale processing, inventory control, and the like. Scanning systems of this general type have been disclosed, for example, in U.S. Pat. Nos. 4,251,798; 4,360,798; 4,369,361; 4,387,297; 4,409,470 and 4,460,120, all of which have been assigned to the same assignee as the instant application.

As disclosed in some of the above patents, one embodiment of such a scanning system resides, inter alia, in a hand-held, portable laser scanning head supported by a user, which is configured to allow the user to aim the head, and more particularly, light beam, at a target and a symbol to be read.

The light source in a laser scanner is typically a gas laser or semiconductor laser. The use of a semiconductor devices as the light source in scanning systems is especially desirable because of their small size, low cost and low power requirements. The laser beam is optically modified, typically by a lens, to form a beam spot of a certain size at the target distance. It is preferred that the beam spot size at the target distance be approximately the same as the minimum width between regions of different light reflectivity, i.e., the bars and spaces of the symbol.

The bar code symbols are formed from bars or elements typically rectangular in shape with a variety of possible widths. The specific arrangement of elements defines the character represented according to a set of rules and definitions specified by the code or "symbology" used. The relative size of the bars and spaces is determined by the type of coding used, as is the actual

size of the bars and spaces. The number of characters per inch represented by the bar code symbol is referred to as the density of the symbol. To encode a desired sequence of characters, a collection of element arrangements are concatenated together to form the complete bar code symbol, with each character of the message being represented by its own corresponding group of elements. In some symbologies a unique "start" and "stop" character is used to indicate where the bar code begins and ends. A number of different bar code symbologies exist. These symbologies include UPC/EAN, Code 39, Code 128, Codabar, and Interleaved 2 of 5.

For the purpose of our discussion, characters recognized and defined by a symbology shall be referred to as legitimate characters, while characters not recognized and defined by that symbology are referred to as illegitimate characters. Thus, an arrangement of elements not decodable by a given symbology corresponds to an illegitimate character(s) for that symbology.

In order to increase the amount of data that can be represented or stored on a given amount of surface area, several new bar code symbologies have recently been developed. One of these new code standards, Code 49, introduces a "two-dimensional" concept by stacking rows of characters vertically instead of extending the bars horizontally. That is, there are several rows of bar and space patterns, instead of only one row. The structure of Code 49 is described in U.S. Pat. No. 4,794,239, which is hereby incorporated by reference.

A one-dimensional single-line scan, as ordinarily provided by hand-held readers, has disadvantages in reading these two dimensional bar codes; that is, the reader must be aimed at each row, individually. Likewise, the multiple-scan-line readers produce a number of scan lines at an angle to one another so these are not suitable for recognizing a Code 49 type of two-dimensional symbols.

In the scanning systems known in the art, the light beam is directed by a lens or similar optical components along a light path toward a target that includes a bar code symbol on the surface. The scanner functions by repetitively scanning the light beam in a line or series of lines across the symbol rising scanning component such as a mirror disposed in the light path. The scanning component may either sweep the beam spot across the symbol and trace a scan line across and past the symbol, or scan the field in view of the scanner, or do both.

Scanning systems also include a sensor or photodetector which functions to detect light reflected from the symbol. The photo-detector is therefore positioned in the scanner or in an optical path in which it has a field of view which extends across and slightly past the symbol. A portion of the reflected light which is reflected off the symbol is detected and converted into an electrical signal, and electronic circuitry or software decodes the electrical signal into a digital representation of the data represented by the symbol that has been scanned. For example, the analog electrical signal from the photodetector may typically be converted into a pulse width modulated digital signal, with the widths corresponding to the physical widths of the bars and spaces. Such a signal is then decoded according to the specific symbology into a binary representation of the data encoded in the symbol, and to the alphanumeric characters so represented.

The decoding process in known scanning systems usually work in the following way. The decoder re-

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ceives the pulse width modulated digital signal from the scanner, and an algorithm implemented in software attempts to decode the scan. If the start and stop characters and the characters between them in the scan were decoded successfully and completely, the decoding process terminates and an indicator of a successful read (such as a green light and/or an audible beep) is provided to the user. Otherwise, the decoder receives the next scan, performs another decode attempt on that scan, and so on, until a completely decoded scan is achieved or no more scans are available.

SUMMARY OF INVENTION

Briefly, and in general terms, the invention provides a system and a method for reading bar code symbols or the like, including a scanner for generating a laser beam directed toward a target producing a narrow raster scanning pattern (or a single scanning line) that enables the user to manually aim and direct the beam to the location desired by the user, and a relatively wider raster scanning pattern (or a dual scanning line) that increases in height and sweeps an entire symbol to be read. The system further includes a detector for receiving reflected light from such symbol to determine whether a valid symbol has been scanned and to produce electrical signals corresponding to data represented by such symbol.

The novel features which are considered as characteristic of the invention are set forth in particular in the appended claims. The invention itself, however, both as to its construction and its method of operation, together with additional objects and advantages thereof, will be best understood from the following description of specific embodiments when read in connection with the accompanying drawings.

BRIEF DESCRIPTION OF DRAWING

FIG. 1 is a highly simplified diagrammatic representation of one embodiment of a laser scanning system according to the present invention;

FIG. 2 is a diagram that depicts the scanning pattern of a raster scanner known in the prior art;

FIG. 3a, 3b, 3c and 3d depict the raster scanning pattern during different time intervals during reading a two dimensional bar code according to the present invention;

FIG. 4 is a pictorial representation of the raster scanning beam traversing a one dimensional bar code;

FIG. 5a, 5b, 5c and 5d is a pictorial representation of the raster scanning beam traversing a two dimensional bar code which is misaligned with respect to the direction of scan, and re-orienting the direction of scan;

FIG. 6 is a schematic diagram of an electrical circuit used to detect the bar and space patterns of a scan in order to determine whether a valid bar code has been scanned;

FIG. 7 is a flow chart of an algorithm according to the present invention to distinguish one and two dimensional bar codes;

FIG. 8 is a block diagram of a circuit utilized in connection with the algorithm according to the present invention;

FIG. 9 is a graph depicting a control signal utilized in connection with the circuit of FIG. 8;

FIG. 10 is a schematic diagram of a circuit for generating the control signal of FIG. 9;

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FIG. 11a, 11b, 11c and 11d depict various types of raster scanning pattern traversing a two dimensional bar code for comparative illustration;

FIG. 12a, 12b, 12c and 12d are graphic representations of the density of scan lines at positions along the y-axis of the scan patterns of FIG. 11a, 11b, 11c and 11d respectively; and

FIG. 13a, 13b, and 13c depicts a dual line scanning pattern that functions in a similar manner to the raster scanning pattern of FIG. 3.

DESCRIPTION OF THE PREFERRED EMBODIMENT

As used in this specification and the following claims, the term "symbol" and "bar code" is intended to be broadly construed and to cover not only patterns composed of alternating bars and spaces of various widths, but also other one or two dimensional graphic patterns, as well as alphanumeric characters.

The invention generally relates to a scanner system based upon light source for reading indicia of different light reflectivity such as bar code symbols. More particularly, the invention provides a scanner system in which adjustment of the spatial coverage of the raster scanning pattern of the scanning beam is automatically made to effect appropriate detection, sweeping and/or scanning of symbols to be read. The invention further provides a method for operating a scanner system by providing a signal to the current drive of the light source and the scan controller in response to detection of indicia which represents or may represent a portion of a desired target, such as a bar code symbol.

The present invention also relates to scanning systems incorporating techniques for automatically initiating and terminating scanning of the target. One feature of some scanner systems is the use of a manually operated trigger to initiate scanning of the target, such as described in U.S. Pat. No. 4,387,297. Although for many applications the use of a trigger is an important feature, there are some applications in which it may be desirable to use alternate activation techniques to initiating scanning, and such techniques are also within the scope of the present invention.

The present invention provides a method and apparatus for operating a scanning system in which two different types of bar codes may be read—a standard linear bar code, and a two-dimensional bar code. The present invention also provides a technique for determining the type of bar code and adjusting the spatial coverage or vertical sweep of the raster scanning beam in order to effect a sufficient sweep to fully read a two dimensional bar code.

Referring to FIG. 1, there is shown a highly simplified embodiment of one type of bar code reader that may be designed according to the principles of the present invention. The reader 100 may be implemented in a hand-held scanner, as illustrated, or a desk-top workstation or stationery scanner. In the preferred embodiment, the arrangement is implemented in a housing 155 that includes a exit port 156 through which an outgoing laser light beam 151 is directed to impinge on, and to be scanned across, symbols 170 located exteriorly of the housing.

This hand-held device of FIG. 1 is generally of the style disclosed in Swartz et al U.S. Pat. No. 4,760,248, or in Symbol Technologies, Inc. U.S. Pat. No. 4,896,026 and also similar to the configuration of a bar code reader commercially available as part number LS 8100

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or LS 2000 from Symbol Technologies, Inc. Alternatively, or in addition, features of Swartz et al U.S. Pat. No. 4,387,297, or Shepard et al U.S. Pat. No. 4,409,470, both such patents assigned to Symbol Technologies, Inc., may be employed in constructing the bar code reader unit of FIG. 1. These U.S. Pat. Nos. 4,760,248, 4,896,026 and 4,409,470 are incorporated herein by reference, but the general design of such devices will be briefly described here for reference.

Turning in FIG. 1 in more detail, an outgoing light beam 151 is generated in the reader 100, usually by a laser diode or the like, and directed to impinge upon a bar code symbol disposed on a target a few inches from the front of the reader unit. The outgoing beam 151 is scanned in a linear raster scan pattern in the present invention, and the user positions the hand-held unit so this scan pattern transverses the symbol to be read. Reflected and/or scattered light 152 from the symbol is detected by a light-responsive device 158 in the reader unit, producing serial electrical signals to be processed and decoded for reproducing the data represented by the bar code. As used hereinafter, the term "reflected light" shall mean reflected and/or scattered light.

In a preferred embodiment, the reader unit 100 is a gun shaped device, having a pistol-grip type of handle 153. A movable trigger 154 is employed to allow the user to activate the light beam 151 and detector circuitry when the user has positioned the device to point at the symbol to be read. A light-weight plastic housing 155 contains the laser light source 146, the detector 158, the optics 157, 147, 159 signal processing circuitry including a detector 17, and the CPU 140 as well as power source or battery 162. A light-transmissive window 156 in the front end of the housing 155 allows the outgoing light beam 151 to exit and the incoming reflected light 152 to enter. The reader 100 is designed to be aimed at a bar code symbol by the user from a position in which the reader 100 is spaced from the symbol, i.e., not touching the symbol or moving across the symbol. Typically, this type of hand-held bar code reader is specified to operate in the range of perhaps several inches.

The reader 100 may also function as a portable computer terminal, and include a keyboard 148 and a display 149, such as described in the previously noted U.S. Pat. No. 4,409,470.

As further depicted in FIG. 1, a suitable lens 157 (or multiple lens system) may be used to focus the scanned beam into the bar code symbol at an appropriate reference plane. A light source 146 such as a semiconductor laser diode is positioned to introduce a light beam into the axis of the lens 157, and the beam passes through a partially-silvered mirror 147 and other lenses or beam-shaping structure as needed, along with an oscillating mirror 159 which is attached to a scanning motor 160 activated when the trigger 154 is pulled. If the light produced by the source 146 is not visible, an aiming light may be included in the optical system. The aiming light, if needed, produces a visible-light spot which may be fixed, or scanned just like the laser beam; the user employs this visible light to aim the reader unit at the symbol.

FIG. 2 is a diagram that depicts the scanning pattern of a raster scanner known in the prior art. Such a pattern may be generated by vertical (or y-direction) displacement of a linear scan line driven in the x-direction, such as described in U.S. Pat. No. 4,387,297. In the prior art such scan pattern is fixed during scanning and reading of the symbol.

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Turning next to FIG. 3, there is shown a sequence of views as a target containing a symbol is scanned by a raster scanning pattern to show the operation of the present invention in one embodiment.

As suggested from the highly simplified discussion in connection with FIG. 3, a feature of the present invention is to provide a circuit which is capable of sampling the reflected light from only one portion of a target and performing a computation or analysis on the detected electrical signal to determine if the detected portion is indicative that a bar code symbol has been detected. The preferred embodiment of such a detector circuit will be subsequently described with reference to FIG. 6.

One technique according to the present invention is to process the electrical signal to produce a test signal to determine if the reflected light of variable intensity represents a spatial variation of different light reflectivity that could be indicative of the presence of a predetermined indicia pattern such as a bar code symbol.

An enabling signal would then be generated if the test signal exceeds a predetermined reference signal. The light beam is then modified in response to the enabling signal in one or more respects as will be subsequently described.

Another technique is to process the electrical signal to produce a count of the number of transitions between portions of different light reflectivity during a predetermined time period. The count would be used to determine whether the reflected light of different light reflectivity is indicative of a presence of a predetermined indicia pattern such as a generic bar code symbol, a class of bar code symbols, or even a specific bar code symbol. An enabling signal would be generated if the count exceeds a predetermined minimum. Again, the light beam would be modified in response to the enabling signal.

Still another technique is to process the electrical signal to compute the ratio of the length of a indicia portion of low light reflectivity to one of high reflectivity to determine whether the reflected light of variable intensity represents a spatial variation of different light reflectivity that could be indicative of a presence of a predetermined indicia pattern and for generating an enabling signal if the ratio is less than a predetermined value. The raster scanning pattern would be modified in response to the enabling signal.

Yet another technique is to process the electrical signal to compare the signal from a first scan with the signal from a second subsequent scan to determine whether the reflected light of variable intensity over successive scans represents a substantially identical spatial variation of different light reflectivity that could be indicative of a presence of a predetermined indicia pattern. An enabling signal is generated if the comparison between a predetermined number of successive scans results in close or nearly identical match. The light beam would be modified in response to the enabling signal. Yet another is to compare several scans to determine if similar scans come in groups, which would be indicative of a 2D bar code.

FIG. 3a is a highly simplified schematic representation of the present invention during a first stage of operation in which a bar code symbol, in this example a two dimensional bar code symbol, is spaced apart from the scanning pattern of the emitted light, depicted as a "narrow" raster scanning pattern. By a "narrow" pattern, as used in this specification, we mean a pattern having a

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height (in the y-direction), much smaller than the length (in the x-direction) of the scanning pattern.

The operation of the present invention begins when a portion of a coded indicia is present in the scanning pattern, i.e. at the second stage shown in FIG. 3b. If the scanner is hand-held, the user, moves the scanner and positions it so that the scanning beam is directed to the location of the coded indicia. A portion of a coded indicia will be present in the scanning pattern of the emitted light from the scanner as shown in FIG. 3b. If the scanner is stationary, the user will move the target into the position of the pattern. The detector circuit 17 shown in FIG. 6 is now operative to detect a portion of a symbol and will generate a laser enable signal to indicate if a bar code has been detected. If no bar code is detected, the user may also seek to vary the distance between the scanner and the target, since the working range of the scanner may be limited even though the scanning pattern illuminates the bar code. The algorithm according to the present invention will further indicate that in this example a two dimensional bar code has apparently been detected, and will shift operation of the apparatus into a third stage of operation.

There are two ways to perform this operation. The first way is to decode the first row and determine on the basis of the decoded information whether the bar code is a one dimensional or two dimensional bar code. The second way is to utilize an intelligent sensing algorithm which is capable of determining on the basis of the code words detected and decoded whether the portion read is a portion of a one dimensional or a two dimensional bar code.

FIG. 3c is a highly simplified schematic representation of the operation of the apparatus of the present invention during a third stage of operation in which the raster scanning pattern has increased in height so that a greater vertical dimension of the bar code is present in the scanning pattern of the emitted light. The bar code rows which are present in the scanning pattern will be read, decoded, and interpreted to determine whether an entire two dimensional bar code symbol has been scanned, as will be subsequently described.

FIG. 3d is a highly simplified schematic representation of the operation of the apparatus of the present invention during a fourth stage of operation after the raster height has increased further and the entire bar code is present in the scanning pattern of the emitted light. After the entire bar code is read and decoded, the raster pattern will be terminated, or alternatively become narrow or compress in height so that only a portion of the indicia will be covered by the beam.

As suggested from the highly simplified discussion in connection with FIG. 3, a feature of the present invention is to provide a circuit which is capable of sampling the reflected light from only a portion of a symbol and performing a computation or analysis to determine if the detected portion is indicative that a one or two dimensional bar code symbol has been detected. According to the embodiment shown in FIG. 3, the result of the computation or analysis by an algorithm may be used to change the raster height between the second and third stages, as well as between the third and fourth stages of operation in the event a two dimensional bar code has been detected.

FIG. 4 depicts the raster scanning pattern traversing a one dimensional bar code, and more particularly a bar code which is skewed or misaligned with respect to the direction of scanning of the scan lines. It is noted by

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inspection of the Figure that even if the scan lines are not orthogonal to the bar code's vertical bars, successive scan lines still read the same sequence of bars and spaces. Such fact is used by the algorithm of the present invention to conclude that a one dimensional bar code has been detected.

FIG. 5a, 5b, 5c and 5d is a pictorial representation of the raster scanning beam traversing a two dimensional bar code which is misaligned with respect to the direction of scan, and depicting the process according to the present invention of reorienting the direction of scan until the scan pattern is aligned with the bar code.

FIG. 5a is a highly simplified schematic representation of the raster scanning pattern of the present invention during a first stage of operation in which the position of the two dimensional bar code is skewed or misaligned with respect to the direction of the raster scanning pattern.

The operation of the present invention begins when the algorithm determines that a skewed bar code is present. Reference is made here to co-pending U.S. patent application Ser. No. 317,433 for a device and method for reading skewed two dimensional bar codes. The circuitry and optical components as described in such application may be utilized in the present invention to reorient the raster scanning pattern, as shown in FIG. 5b. Further analysis is performed on data received from the new orientation, and if it is determined the pattern is still skewed, the scanning pattern will again be reoriented in an interactive process until it is finally aligned with the bar code as shown in the position of FIG. 5c.

FIG. 5c is a highly simplified schematic representation of the operation of the apparatus of the present invention during a third stage of operation in which the raster scanning pattern has been reoriented so that the rows of the two dimensional bar code are parallel to the scanning pattern. The bar code rows which are present in the scanning pattern will be read, decoded, and interpreted, as described in connection with FIG. 3b.

FIG. 5d is a highly simplified schematic representation of the operation of the apparatus of the present invention during a fourth stage of operation after the raster height has increased further and the entire bar code is present in the scanning pattern of the emitted light. After the entire bar code is read and decoded, the raster pattern will narrow so that only a portion of the indicia will be covered by the beam.

The present invention also permits a relatively bright, small height rectangular laser raster scanning pattern to be used to enable the user to aim and direct the beam toward a bar code symbol to be read. The user then proceeds to scan the symbol, and the device detects light reflected from the symbol and generating an electrical signal in response to the reflected light. The signal is processed and interpreted, and control circuitry modifies the height of the raster scan pattern in response to the electrical signal.

The aiming and scanning feature using a small height raster scanning pattern may be implemented with different activation or triggering modes, which has been described in detail in previous applications.

There are a number of possible scanning modes that may be implemented in connection with hand-held or fixed mount laser scanners: (a) the normal trigger mode; (b) the trigger spot and scan mode; and (c) the dual position trigger mode. Modes (a) and (b) have been described in U.S. patent application Ser. No. 349,860 filed May 10, 1989, in connection with an integrated

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scanning terminal, but such scanning modes are equally applicable to other types of scanners. Mode (c) has been described in U.S. patent application Ser. No. 544,628, filed Jun. 27, 1990, in connection with a long-range scanner, and again such a scanning mode is equally applicable to other types of scanners.

In the normal triggered mode, the laser beam is normally off. A trigger is used in the normal triggered mode to initiate the rapid and repetitive scanning of the target symbol. For proper counting, it is necessary to distinguish between the situation in which many scans have been performed on a single object, or the situation in which one or more scans have been performed on a plurality of objects with identical symbols. The capability of sensing each object to be scanned in its turn is critical for successful applications of bar code scanning in data collection, inventory, and similar applications.

As is known in prior art scanners (such as described in U.S. Pat. No. 4,387,297) a trigger is operative for actuating the scanning means to repetitively sweep the bar code symbol a number of times each time the trigger is actuated. The trigger is preferably a manually-depressible switch mounted on the housing in the vicinity of the interconnection of the barrel and handle portions of the housing. The trigger switch is located on the handle portion such that the forefinger of the user's hand can be used to manually depress the switch. Each time the switch is depressed the scanning means sweeps the symbol many times, until a complete decode or a time out is reached.

In the normal triggered mode, when the decode circuitry successfully decodes the symbol, the decode circuitry generates a successful decode signal and may actuate an indicator located in the scanner. The indicator may be an auditory-type beeper and/or a light emitting diode. When the beeper sounds and/or when the diode lights up, then the user knows that the scanning for that particular symbol has been terminated.

In the triggered spot and scan mode, such as described in U.S. Pat. No. 4,933,538, after the trigger is pulled, the beam only comes on at a narrow scanning angle. In such an operational mode, a very bright, short line about 1" in length is formed by the narrow laser scanning beam. The bright small line formed on the target is used by the user holding the laser scanner to manually aim and direct the beam to the specific location on the target where the user actually sees the bar code is located. When an indicia pattern indicative of a bar code symbol has been detected, the beam will automatically widen, thereby sweeping the entire symbol so that it can be decoded.

In the dual position trigger mode, the trigger has a first and a second operational position. If the trigger is pulled to a first position the beam is directed in a fixed, non-scanning path to form a narrow aiming beam. In such an operational mode, a very bright spot is formed by the narrow beam. The bright spot is used by the user holding the laser scanner to manually aim and direct the beam to the location where the user actually sees the bar code is located. Typically the user will position the spot approximately at the center of the bar code. The user will then pull the trigger to a second position to initiate scanning. When the second position of the trigger is reached, the beam will widen to sweep the entire symbol so that it can be decoded. Although in some cases the beam may be dimly reflective or not visible to the user, since the beam has already been positioned, the

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sweep will cover the symbol and decode will take place.

In the present invention, the narrow scanning pattern will function as an aiming beam and as a range orientation detector. The pattern will not open up unless it is within the proper range and orientation, thus providing a method for teaching the operator the right orientation of a hand-held scanner to correctly read a bar code symbol.

Turning next to FIG. 6 there is shown a schematic diagram of an embodiment of a preferred detector circuit 17 as used in the present invention. This circuit is identical to that disclosed in parent application Ser. No. 506,674, and is included herein for completeness of the discussion. The signal from the amplifier/digitizer 16 is applied to the input of an inverter 31, the output of which is applied to a diode 32. An RC circuit is formed by first resistor R1 and capacitor (C) 34 with the resistor R1 being connected in series between the output of the diode 32 and a first input 35 of an open collector output comparator 37. The capacitor (C) 34 is connected between the first input 35 and ground potential, and a second resistor R2 is connected between first input 35 and ground potential. The resistance value of resistor R1 is preferably much less than that of resistor R2.

The second input 38 of the comparator is connected to the node of a voltage divider formed by resistor R4 and R5 which are connected in series between potential V and ground. The output 41 of the comparator 37 is connected to the "laser enable" signal line, as well as a feedback through a resistor R3. The feedback from the output of the comparator 37 provides a hysteresis effect to the comparison operation since the other terminal of the resistor R3 is connected to the second input 38 of the comparator 37. The operation of the detector circuit 17 can be described as follows: when the digitizer outputs a bar, the capacitor charges up with a time constant of approximately $R1C$ since R2 is much greater than R1. When the digitizer outputs a space, the capacitor discharges through R2 since the diode 32 prevents the discharging through R1. The time constant $R2C$ is much greater than the time constant $R1C$ so that more space time is required to cancel the effect of a bar.

After several bars and spaces of typical density, a voltage is developed on the capacitor 34 which exceeds the threshold which has been set with the use of the comparator 37. At this time, a "trigger" or laser enable signal is output from the comparator 37 to indicate the presence of a bar code.

The open collector output of the comparator 37 is driven low at this point which lowers the threshold of the comparator so that minor voltage changes on the capacitor 34 due to the following bars and spaces, and quite zone, will not disable the trigger signal.

The circuit as described would also trigger if a long black bar were to be scanned. However, in the preferred embodiment, the digitizer includes a circuit which discriminates against reading a long black bar, i.e., the digitizer functions as a high pass filter. One such digitizer circuit would utilize a time-out so that if a long black bar was scanned, only a short pulse would be generated. When such a short pulse signal is applied to the detector circuit 16, the threshold will not be exceeded and a "trigger" signal will not be output.

The trigger signal will only be released after a relatively long time during which there are no bars digitized. When the scanner is moved away from a symbol, the capacitor will discharge through $R2C$ and the trig-

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ger signal will be released which will indicate to the decoding logic or the detector circuit that the same symbol is no longer being scanned.

The bar code sensing circuit depicted in FIG. 6 is one of the key features of the present invention since it is sensitive to a specific pattern of images. The circuitry is responsive to a pattern of high and low reflected light signals to charge and discharge the capacitor 34, with the net charge being utilized to generate a trigger signal after some threshold has been reached.

The circuit described in FIG. 6 is useful to discriminate a barcode in the scan field from text or other graphics. To do this it exploits the single dimension aspect of a bar code to differentiate it from text. It relies on motion of the scanline, which ordinarily would come from motion of the scanner in a user's hand, to compare different slices of the pattern in the scanfield. If successive slices were similar, within limits determined by the implementation, it was highly probable that a barcode was being scanned. If successive slices were dissimilar, it was likely that a barcode was not being scanned. This algorithm is modified to allow two dimensional bar code to be discriminated from graphics. It relies on the one dimensional nature within regions in the Y dimension.

The algorithm according to the present invention is preferably implemented in software, and executed by the CPU 140 in the scanner. The algorithm in FIG. 7 can be used to discriminate among one dimensional bar codes, two dimensional (or "stacked") barcodes, and text or other graphics. A raster scan pattern according to the present invention is naturally suited to work together with this algorithm since it automatically provides the movement of the scanline orthogonal to the scanline orientation, which guarantees that successive scanlines cross different slices of the scanned pattern, which is relied upon by the algorithm.

The algorithm minimizes the amount of processing that would be done on the data provided from the scanner, and thus reduces the latency the system will have in fully reading a barcode symbol. Another feature of the algorithm is to provide a method to control operational parameters of the scanning system, such as horizontal and vertical scan angles, in response to the type of barcode that is determined to be scanned.

FIG. 7 illustrates an operational flow chart for a scanner operative for scanning along a predetermined direction lengthwise of an indicium, e.g., a bar code symbol, to be read (also known as X-axis scanning), and for scanning in a transverse direction which is orthogonal to the predetermined direction (also known as Y-axis scanning). As described, for example, in U.S. Pat. No. 4,387,297, individual X-axis scan means and Y-axis scan means may be utilized to obtain a scan pattern of any desired shape. Thus, if the X-axis scan means is solely operated, then only a generally linear scan line will be generated at the symbol. If the X- and Y-axis scan means are driven at uniform rates of speed, then a raster-type scan pattern, comprising a set of generally parallel scan lines, extending both along the length and height of the symbol, will be generated. If the X- and Y-axis scan means are driven at sinusoidally varying rates of speed, then a Lissajous-type omnidirectional scan pattern is generated at the symbol. Reference is also made to U.S. patent application Ser. No. 520,464, filed May 8, 1990, incorporated herein by reference.

Of course, other types of scan patterns are comprehended within the spirit of this invention. For example,

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the X-axis scan means need not generate a single scan line as the X-axis scan pattern, but, instead, can generate a plurality of mutually parallel scan lines as the X-axis scan pattern. This plurality of scan lines is spaced apart over a relatively short first distance across the height of the symbol. In the case where the Y-axis scan pattern is also constituted of a set of mutually parallel scan lines spaced apart of each other over a second distance along the height of the symbol, this second distance is greater than the first distance. Thus, a variety of raster-type scan patterns may be generated, one of reduced height to representing X-axis scanning, and others of various increased heights to which represent Y-axis scanning.

As used in FIG. 7, the term "Y opening" signifies operation of the Y-axis scan means to change the height of the raster scanning pattern.

Block 200 in FIG. 7 represents the first step in the algorithm at the onset of scanning. In the case of a hand-held scanner, scanning is typically initiated by mutual actuation of a trigger. There is either no Y-axis scanning or a constant y-axis scanning at this time, i.e., no increase in height of the raster scanning pattern.

Block 201 represents the next sequential step in the algorithm corresponding to operation of the X-axis scan means and acquiring the data resulting from a single scan across the target.

Block 202 represents the next step of the above-mentioned algorithm for distinguishing between one-dimensional and two-dimensional bar code symbols. If the algorithm determines that the symbol is not two dimensional, then block 203 represents the attempted decoding of the one-dimensional symbol. If the one-dimensional symbol is successfully decoded at the stage of block 205, then the decoded data is sent out to the next stage at block 207 for further processing. If the one-dimensional symbol is not successfully decoded at block 205, then the X-axis scan means at block 201 remains operational until a successful decode has occurred, or until a predetermined amount of time has elapsed.

If the algorithm at block 202 determines that the symbol is two dimensional, then the Y-axis scan means is actuated at block 204. Block 206 represents the attempted decoding of the two-dimensional symbol. If the two-dimensional symbol is successfully decoded at block 208, then the decoded data is sent out at block 209 for further processing and, concomitantly, the Y-axis scan means is deactuated. If the two-dimensional symbol is not successfully decoded at block 208, then the Y-axis scan means remains operational until a successful decode has occurred or until a predetermined amount of time has elapsed. The predetermined amount of time is typically on the order of three (3) seconds, which would be regarded as sufficient time for an operator to sight the symbol and obtain a successful decode.

Turning now to FIG. 8, in a preferred embodiment, the aforementioned block 202 which depicts the algorithm for distinguishing between one-dimensional and two-dimensional bar code symbols generates a digital output signal which has either a HIGH state or a LOW state, depending on whether a one-dimensional or a two-dimensional symbol is respectively detected. This output signal is conducted to an amplitude control circuit 210 (shown in detail in FIG. 10) operative for generating a control signal V_c (shown graphically as a function of time in FIG. 9).

Returning to FIG. 8, an oscillator 212 for Y-axis operation is operative for generating the basic driving

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signal for operating a Y-axis driver 214 and, in turn, the y-axis scan means 216. The driving signal has a periodic waveform, e.g. sinusoidal, triangular, etc. The driving signal is fed together with the control signal V_c to a multiplier 218. The control signal V_c controls the amplitude of the driving signal.

Another important feature of the invention is to teach the operator of the scanner the correct range and orientation of the scanner to read symbols quickly and accurately, so a feed-back signal (an audible "beep" or a visual indicator) may be actuated when a symbol is detected in range.

As previously described, the preferred embodiment generates a first raster-scan of reduced height for x-axis scanning and a second raster-scan of increased height for Y-axis scanning. In this case, when a two-dimensional bar code symbol is detected, the height or Y dimension of the raster pattern grows linearly from an initial amplitude V_1 until the final height of amplitude V_2 is reached. The control signal needed to perform this operation is depicted in FIG. 9.

FIG. 10 shows the amplitude control circuit 210 operative for generating the control signal V_c depicted in FIG. 9. The output signal from block 202 is conducted to an analog switch 220 which is connected in parallel across a charging capacitor C_1 .

An OP Amp 222 has one input connected to ground through a variable resistor R_8 , and another input connector to a junction between resistor R_6 and R_7 . The other end of resistor R_6 is connected to a supply voltage V_{cc} , and the other end of resistor R_7 is connected to ground. A voltage V_j appears at the junction between resistors R_6 and R_7 .

The output of the OP Amp 222 is connected to a zener diode through a resistor R_9 , and also to one side of the capacitor C_1 . An output voltage V_o is connected to ground through a potentiometer R_{10} from whose wiper arm the control voltage V_c is taken.

In operation, when no two-dimensional bar code symbol is detected, the output signal from block 202 is set to the LOW state, thereby closing the switch 220 and discharging C_1 . V_o is then equal to V_j which is set by V_{cc} , R_6 and R_7 .

When a two-dimensional bar code symbol is detected, the output signal of block 202 is set to the HIGH state, which opens the switch 220, thereby allowing C_1 to charge up at a rate set by V_j , R_8 and C_1 . During this time, the circuit is operating as a linear integrator and the voltage V_o will grow linearly. Eventually, V_o will reach the breakdown voltage V_z of the zener diode and will no longer rise. R_9 limits the current in the zener diode to safe levels. The voltage V_o will then remain at the voltage V_z until the switch 220 is again closed which will rapidly discharge C_1 and force V_o to be equal to V_j . R_{10} is provided to scale the voltage V_o to the desired voltage V_c .

The above described method of operation assures that the scan line is positioned by the user at approximately the vertical midpoint of the bar code. An additional embodiment of the present invention contemplates that a user may not in fact accurately position the scan line at the midpoint, but closer to the top or bottom edge. Such an embodiment provides an algorithm to determine how the raster scan is implemented in both the y-positive and y-negative directions; for example, the raster scan may grow in the positive and negative directions at different rates depending upon the position along the y-axis of the initial scan line. The position of

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the initial scan line may be determined by reading the row number of the 2D bar code, and an algorithm employed to determine whether the most efficient expansion of the raster scan pattern would be to row the pattern at different rates on either side of the initial scan line. As an example, if the initial scan line was determined to be on the third row of a 2D bar code the algorithm would specify that the growth of the raster scan pattern in the y-negative direction would be considerably greater than the growth in the y-positive direction. The implementation of such an algorithm and the specification of the growth rates based upon the row of the initial scan line are all within the skill of those familiar with the art of decoding 2D bar code symbols and will not be described in detail here.

Turning next to FIG. 11 and 12, there is shown a sequence of views in FIG. 11 as a target containing a symbol is scanned by a raster scanning pattern to show the offset of the operation of the present invention in terms of the line density (shown in FIG. 12) as different stages of operation corresponding to an increasing height.

FIG. 11a is a highly simplified schematic representation of the integration over time of the scanning patterns of FIGS. 11b, 11c, and 11d resulting in average uniform density. The line density (or number of raster scanning lines per unit vertical dimension) is shown graphically in FIG. 12a immediately to the right of FIG. 11a.

FIG. 11b is a highly simplified schematic representation of the scanning pattern of the raster scanning pattern embodiment of the present invention during a first stage of operation in which a bar code symbol, in this example a two dimensional bar code symbol, is contained within the scanning pattern of the emitted light. FIG. 12b depicts the line density of the scanning pattern shown in FIG. 11b.

FIG. 11c is a highly simplified schematic representation of the operation of the apparatus of the present invention during a second stage of operation in which the raster scanning pattern has increased in height so that a greater vertical dimension of the bar code is present in the scanning pattern of the emitted light. The bar code rows which are present in the scanning pattern will be read, decoded, and interpreted to determine whether an entire two dimensional bar code symbol has been scanned, as has been previously described. FIG. 12c depicts the line density of the scanning pattern shown in FIG. 11c.

FIG. 11d is a highly simplified schematic representation of the operation of the apparatus of the present invention during a third stage of operation in which the raster pattern height has increased further and the first and last row of the two dimensional bar code is present in the scanning pattern of the emitted light. After the entire bar code is read and decoded, the raster pattern will be terminated, or alternatively become narrow height. FIG. 12d depicts the line density of the scanning pattern shown in FIG. 11d.

Turning next to FIG. 13, there is shown a sequence of views as a target containing a symbol is scanned by a dual line scanning pattern to show the operation of the present invention in another embodiment.

FIG. 13a is a highly simplified schematic representation of scanning pattern of the dual line embodiment of the present invention during a first stage of operation in which a bar code symbol, in this example a two dimensional bar code symbol, is contained within the scanning

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pattern of the emitted light, two lines spaced a narrow distance apart.

There are different methods for generating a dual line scanning pattern. The first method is to utilize two laser with each laser associated with one of the two line scanning patterns. Each laser could be alternately activated so that at any given time only one laser beam is directed to the target. A second approach is to utilize a single laser with any optical mechanism which scans the target alternately with one of the two scan lines and then the other. Such an optical mechanism may be, for example, a scanning mirror which is tilted between two angles. Each position of such mirror corresponds to one of the scanning paths. The angle may be increased over time so that the distance between the two scan lines increases from any initial value to a maximum value. Reference may be made to U.S. Pat. No. 4,871,904 to disclose the use of two mirrors in any optical path for changing a scan pattern. In the implementation section in the present invention the two mirrors are not operated simultaneously, but the mirror is shifted between two fixed positions and only when such mirror has been placed in such fixed position would the second mirror associate with the longitudinal scanning line being activated.

As has been discussed in connection with FIG. 3, the detector circuit 17 is now operative to detect a portion of a symbol and functions to generate a laser enable signal if a bar code has been detected. The algorithm according to the present invention will further indicate that in this example a two dimensional bar code has apparently been detected, and will shift operation of the apparatus into the next stage of operation.

FIG. 13b is a highly simplified schematic representation of the operation of the apparatus of the present invention during a second stage of operation after the dual scanning pattern has increased in height so that a greater vertical dimension of the bar code is present in the scanning pattern of the emitted light. The bar code rows which are present in the scanning pattern will be read, decoded, and interpreted to determine whether an entire two dimensional bar code symbol has been scanned, as has been previously described.

FIG. 13c is a highly simplified schematic representation of the operation of the apparatus of the present invention during a third stage of operation after the dual line height has increased further and the first and last rows or the two dimensional bar code is present in the scanning pattern of the emitted light. After the entire bar code is read and decoded, the dual line pattern will be terminated, or alternatively become narrow height.

Although the present invention has been described with respect to reading one or two dimensional bar codes, it is not limited to such embodiments, but may also be applicable to more complex indicia scanning applications. It is conceivable that the method of the present invention may also find application for use with various machine vision or optical character recognition applications in which information is derived from other types of indicia such as characters or from the surface characteristics of the article being scanned.

In all of the various embodiments, the elements of the scanner may be assembled into a very compact package that allows the scanner to be fabricated as a single printed circuit board or integral module. Such a module can interchangeably be used as the laser scanning element for a variety of different types of data acquisition systems. For example, the module may be alternately

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used in a hand-held scanner, a table top scanner attached to a flexible arm or mounting extending over the surface of the table or attached to the underside of the table top, or mounted as a subcomponent or subassembly of a more sophisticated data acquisition system.

The module would advantageously comprise a laser/optics subassembly mounted on a support, a scanning element such as a rotating or reciprocating mirror, and a photodetector component. Control or data lines associated with such components may be connected to an electrical connector mounted on the edge or external surface of the module to enable the module to be electrically connected to a mating connector associated with other elements of data acquisition system.

An individual module may have specific scanning or decoding characteristics associated with it, e.g. operability at a certain working distance, or operability with a specific symbology or printing density. The characteristics may also be defined through the manual setting of control switches associated with the module. The user may also adapt the data acquisition system to scan different types of articles or the system may be adapted for different applications by interchanging modules on the data acquisition system through the use of the simple electrical connector.

The scanning module described above may also be implemented within a self-contained data acquisition system including one or more such components as keyboard, display, printer, data storage, application software, and data bases. Such a system may also include a communications interface to permit the data acquisition system to communicate with other components of a local area network or with the telephone exchange network, either through a modem or an ISDN interface, or by low power radio broadcast from the portable terminal to a stationary receiver.

It will be understood that each of the features described above, or two or more together, may find a useful application in other types of scanners and bar code readers differing from the types described above.

While the invention has been illustrated and described as embodied in it is not intended to be limited to the details shown, since various modifications and structural changes may be made without departing in any way from the spirit of the present invention.

Without further analysis, the foregoing will so fully reveal the gist of the present invention that others can readily adapt it for various applications without omitting features that, from the standpoint of prior art, fairly constitute essential characteristics of the generic of specific aspects of this invention and, therefore, such adaptations should and are intended to be comprehended within the meaning and range of equivalence of the following claims.

What is claimed is:

1. A device for reading bar code symbols of the like, comprising:

- a) a light source for generating a light beam directed toward a symbol to be read in the form of a raster scan pattern of adjustable height;
- b) a light detector receiving reflected light from said symbol and generating electrical signals responsive to said reflected light;
- c) a control circuit for modifying the height of said scan pattern in response to said electrical signals from said detector.

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2. A device according to claim 1, wherein said control circuit is responsive to whether said electrical signals represent a pattern of a bar code symbol.

3. A device according to claim 2, wherein said control circuit switches said raster scan pattern from a narrow height mode to a wide height mode when said electrical signals represent the pattern of a bar code.

4. A bar code reader comprising:

a) a light beam scanner generating a light beam directed toward a symbol to be read and moving said light beam along said symbol in a raster scanning pattern;

b) a light detector receiving reflected light from said symbol and generating electrical signals responsive to said reflected light;

c) means for controlling the height and/or path shape of said scanning pattern in response to said electric signals.

5. A device according to claim 4, wherein said light beam scanner moves said light beam on a first scan path or on a second scan path in response to said electrical signals.

6. A device according to claim 5, wherein said first and second scan paths are selected by changing the scanning angle of said raster scanning pattern.

7. A method of scanning bar code symbols or the like comprising the steps of:

a) providing a relatively bright, narrow rectangular laser raster scanning pattern for enabling the user to aim and direct the beam toward a bar code symbol to be read;

b) scanning said symbol;

c) detecting light reflected from the symbol and generating an electrical signal in response to said reflected light; and

d) modifying the height of said raster scan pattern in response to said electrical signal.

8. A method according to claim 7, wherein said step of modifying includes changing the shape of said path.

9. A method according to claim 8, further including the step of determining whether or not said electrical signal exhibits characteristics of a bar code signal, and wherein said step of modifying is performed in response to the results from said step of determining.

10. A method according to claim 9 wherein said step of determining includes deciding whether said bar code symbol is a one dimensional or a two dimensional bar code symbol.

11. A method according to claim 9, wherein said step of determining includes deciding if the scanning direction is substantially orthogonal to the bars of a two dimensional bar code symbol.

12. A scanner for electro-optically reading coded indicia that may include linear bar code symbols, or two dimensional symbologies in which data or information is represented in the form of bars or elements of various widths arrayed in rows, one row adjacent to and beneath another row, with each row including a plurality of codewords of information, and each codeword representing at least one information bearing character, comprising:

a) means for directing light from a laser in a pattern of scanning lines at an indicium to be read for reflection therefrom;

b) means for detecting at least a portion of the light reflected from the indicium;

c) means for determining from the reflected light from successive scanning lines whether the indi-

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cium is a portion of a linear bar code symbol, or a portion of a two dimensional symbology; and
d) means for decoding the indicium.

13. A system for reading bar code symbols or the like, comprising

scanning means for generating a laser beam directed toward a target and producing a first scanning pattern that enables the user to manually aim and direct the beam to the location desired by the user and a relatively larger second scanning pattern in the form of a raster that sweeps an entire symbol to be read,

means for changing from said first scanning pattern to said second scanning pattern, and

detection means for receiving reflected light from such symbol to produce electrical signals corresponding to data represented by such symbol.

14. A system according to claim 13 wherein said scanning means includes a semiconductor laser light source to produce said laser beam, and further comprising a gun-shaped housing having an exit port, wherein said scanning means and said detection means are located in said housing, and said housing includes a handle portion of a size designed to be gripped by a user, and a barrel portion connected to the handle portion so as to enable the user to manually aim and direct the laser beam to the target.

15. A system as defined in claim 14, further comprising manually actuatable trigger means on said housing for initiating said first scanning pattern, and indicator means to inform the user that the housing is positioned in the correct working range for reading a bar code symbol.

16. The system as defined in claim 15, wherein said trigger means includes a multi-purpose trigger operatively connected to the scanning means to select between the first scanning pattern and the relatively larger second scanning pattern.

17. The system as defined in claim 13, wherein said symbol includes at least two rows of bar patterns, and said relatively larger second scanning pattern covers the entire symbol with at least two scan lines per row of bar patterns during the reading operation.

18. A system as defined in claim 13, wherein the number of scan lines in said first scanning pattern is substantially equal to the number of scan lines in said relatively larger second scanning pattern.

19. A system as defined in claim 13, wherein said means for initiating said second scanning pattern occurs at a predetermined time after said first scanning pattern is initiated.

20. A system as defined in claim 13, wherein said means for changing to said second scanning pattern is activated if said detection means recognizes that a bar code is being scanned.

21. A system as defined in claim 13, wherein said means for changing to said second scanning pattern is activated if the position of the system is within the proper range and orientation with respect to the symbol.

22. A system as defined in claim 13, wherein said first scanning pattern is a raster scanning pattern.

23. A system for reading bar code symbols or the like, comprising scanning means for generating a laser beam directed toward a target producing a first scanning pattern that has a reflectivity on the target that enables the user to manually aim and direct the beam to the location desired by the user on the target and a sequence of different subsequent scanning patterns that each pro-

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gressively increase height including a scanning pattern that sweeps the entire symbol to be read, and detection means for receiving reflected light from such symbol to produce electrical signals corresponding to data represented by such symbol.

24. A system as defined in claim 23, wherein said first and subsequent scanning patterns are raster scanning patterns.

25. A system as defined in claim 23, wherein said system is hand-held.

26. A system for reading bar code symbols or the like, comprising

scanning means for generating a laser beam directed toward a target and producing a first raster scanning pattern for a first period of time and subsequently a relatively larger

second raster scanning pattern that sweeps the entire height of a symbol to be read;

means for changing the scanning pattern from said first to said second pattern, and

detection means for receiving reflected light from such symbol to produce electrical signals corresponding to data represented by such symbol.

27. The system as defined in claim 26, wherein said symbol includes at least two rows of bar patterns, the number of scan lines in said first scanning pattern is substantially equal to the number of scan lines in said relatively larger second scanning pattern, and

said relatively larger second raster pattern covers the entire symbol with at least two scan lines per row of bar patterns during the reading operation.

28. A system as defined in claim 26, wherein a sequence of larger raster scanning patterns is produced commencing at a predetermined time after said first scanning pattern.

29. A system for reading bar code symbols or the like, comprising

scanning means for generating a laser beam directed toward a target and producing a sequence of two-dimensional different scanning patterns that initially enables the user to position the beam to scan in a direction corresponding to the rows of a multidimensional bar code symbol, and

detection means for receiving reflected light from such symbol from successive scanning patterns to produce electrical signals corresponding to data represented by such symbol until each of said rows of said symbol has been read.

30. A system according to claim 29, wherein said scanning means includes a semiconductor laser light source to produce said laser beam, and further comprising a gun-shaped housing having an exit port, wherein said scanning means and said detection means are located in said housing, and said housing includes a handle portion of a size designed to be gripped by a user, and a barrel portion connected to the handle portion so as to enable the user to manually aim and direct the laser beam to the target.

31. A system as defined in claim 29, further comprising manually actuable trigger means on said housing for initiating a first scanning pattern, and indicator means to inform the user that the housing is positioned in the correct working range for reading a bar code symbol.

32. The system as defined in claim 31, wherein said trigger means includes a multi-position trigger operatively connected to the scanning means to select be-

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tween a first scanning pattern and a second scanning pattern.

33. The system as defined in claim 29, wherein said multidimensional bar code symbol includes at least two rows of bar patterns, and at least one of said scanning patterns covers the entire symbol with at least two scan lines per row of bar patterns.

34. A system as defined in claim 29, wherein the number of scan lines in said first scanning pattern is substantially equal to the number of scan lines in a second scanning pattern.

35. A system as defined in claim 29, wherein said means for initiating a second scanning pattern occurs at a predetermined time after said first scanning pattern is initiated.

36. A system as defined in claim 29, wherein said means for changing to said second scanning pattern is actuated if said detection means recognizes that a bar code is being scanned.

37. A system as defined in claim 29, wherein said means for changing to said second scanning pattern is actuated if the position of the system is within the proper range and orientation with respect to the symbol.

38. In a scanning system for reading bar codes, means for initially scanning at least a portion of the bar code to be read to detect if the same is a linear or multidimensional code and generating a signal indicative thereof, and means actuated by said signal for adjusting the scan pattern to scan such detected type of code.

39. The system as defined in claim 38, wherein said multidimensional bar code symbol includes at least two rows of bar patterns, and the adjusted scan pattern covers the entire symbol with at least two scan lines per row of bar patterns.

40. A system as defined in claim 38, wherein said means for adjusting the scan pattern is actuated if said detection means recognizes that a multidimensional bar code is being scanned.

41. A system as defined in claim 38, wherein said means for adjusting the scan pattern is actuated if the position of the system is within the proper range and orientation with respect to the symbol.

42. A method for electro-optically reading light-reflective indicia, comprising:

scanning means for projecting light on an indicium to be read, and for scanning the indicium with a scan pattern having a first scan characteristic;

detector means for detecting a portion of the light reflected off the indicium, and for generating electrical signals indicative of the detected light portion;

processor means for processing the electrical signals to determine whether the indicium has a predetermined feature; and

changing means responsive to the determination that the indicium has the predetermined feature, for changing the scan pattern to have a different, second characteristic.

43. A system as defined in claim 42, wherein the predetermined feature of the indicium is that the indicium is a one-dimensional bar code symbol having parts of different light reflectivity arranged along a row.

44. A system as defined in claim 42, wherein the predetermined feature of the indicium is that the indicium is a two-dimensional bar code symbol having parts of different light reflectivity arranged along multiple rows.

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45. A system as defined in claim 42, wherein the indicium is a bar code symbol having parts of different light reflectivity arranged along a scan direction, and having a symbol height extending along a transverse direction normal to the scan direction; and wherein the first scan characteristic has a reduced scan height less than said symbol height along the transverse direction; and wherein the second scan characteristic has an enlarged scan height greater than said symbol height along the transverse direction.

46. A system as defined in claim 42, wherein the indicium is a bar code symbol having parts of different light reflectivity arranged along a scan direction; and wherein the first scan characteristics extends along a first axis inclined relative to the scan direction; and wherein the second scan characteristics along a second axis generally parallel to the scan direction.

47. A method system for reading bar code symbols or the like, comprising the steps of:

generating a laser beam directed toward a target and producing a first scanning pattern that enables the user to manually aim and direct the beam to the location desired by the

user and a relatively larger second scanning pattern in the form of a raster that sweeps an entire symbol to be read,

changing from said first scanning pattern to said second scanning pattern, and

receiving reflected light from such symbol to produce electrical signals corresponding to data represented by such symbol.

48. A system as defined in claim 47, further comprising the step of actuating trigger means on a housing for initiating said first scanning pattern.

49. A method for reading bar code symbols or the like, comprising the steps of generating a laser beam directed toward a target and producing a first scanning pattern that has a reflectivity on the target that enables the user to manually aim and direct the beam to the location desired by the user on the target, generating a sequence of different subsequent scanning patterns that each progressively increase in height including a scanning pattern that sweeps the entire symbol to be read, and receiving reflected light from such symbol to produce electrical signals corresponding to data represented by such symbol.

50. The method as defined in claim 49, further comprising the step of actuating a multi-purpose trigger to select between the first scanning pattern and a subsequent scanning pattern.

51. The method as defined in claim 49, wherein said target includes a bar code symbol with at least two rows of bar patterns, and one of said subsequent scanning patterns covers the entire symbol with at least two scan lines per row of bar patterns during the reading operation.

52. A system as defined in claim 49, wherein the number of scan lines in said first scanning pattern is substantially equal to the number of scan lines in said subsequent scanning patterns.

53. A method as defined in claim 49, wherein a second scanning pattern is initiated at a predetermined time after said first scanning pattern is initiated.

54. A method as defined in claim 49, wherein a second scanning pattern is initiated if a bar code is being scanned.

55. A method as defined in claim 49, wherein a second scanning pattern is initiated if the location of the

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scanning system is within the proper range and orientation with respect to the symbol on the target.

56. A method for reading bar code symbols or the like, comprising the steps of

generating a laser beam directed toward a target and producing a first raster scanning pattern for a first period of time and subsequently a relatively larger second raster scanning pattern that sweeps the entire height of a symbol to be read;

changing the scanning pattern from said first to said second pattern, and

receiving reflected light from such symbol to produce electrical signals corresponding to data represented by such symbol.

57. The method as defined in claim 56, wherein said symbol includes at least two rows of bar patterns, the number of scan lines in said first scanning pattern being substantially equal to the number of scan lines in said relatively larger second scanning pattern, and

said relatively larger second raster pattern covers the entire symbol with at least two scan lines per row of bar patterns during the reading operation.

58. A method as defined in claim 56, wherein a sequence of larger raster scanning patterns is produced commencing at a predetermined time after said first scanning pattern.

59. A method as defined in claim 56, wherein said step of changing the scanning pattern is initiated if a bar code is being scanned.

60. A method as defined in claim 56, wherein said step of changing said scanning pattern is initiated if the position of the system is within the proper range and orientation with respect to the symbol.

61. A method for reading bar code symbols or the like, comprising

generating a laser beam directed toward a target and producing a sequence of two-dimensional different scanning patterns that initially enables the user to position the beam to scan in a direction corresponding to the rows of a multi-dimensional bar code symbol, and

receiving reflected light from such symbol from said sequence of scanning patterns to produce electrical signals corresponding to data represented by such symbol until each of said rows of said symbol has been read.

62. The method as defined in claim 61, further comprising the step of actuating a multi-purpose trigger to select between the first scanning pattern and a subsequent scanning pattern.

63. The method as defined in claim 61, wherein said target includes a bar code symbol with at least two rows of bar patterns, and one at said subsequent scanning patterns covers the entire symbol with at least two scan lines per row of bar patterns during the reading operation.

64. A system as defined in claim 61, wherein the number of scan lines in said first scanning pattern is substantially equal to the number of scan lines in said subsequent scanning patterns.

65. A system as defined in claim 61, wherein a second scanning pattern is initiated at a predetermined time after said first scanning pattern is initiated.

66. A system as defined in claim 61, wherein a second scanning pattern is initiated if a bar code is being scanned.

67. A system as defined in claim 61, wherein a second scanning pattern is initiated if the location of the scan-

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ning system is within the proper range and orientation with respect to the symbol on the target.

68. A method of scanning bar code symbols or the like comprising the steps of:

- a) providing a relatively bright scanning pattern for enabling the user to aim and direct the beam toward a bar code symbol to be read;
- b) scanning said symbol;
- c) detecting light reflected from the symbol and generating an electrical signal in response to said reflected light; and
- d) modifying the scanning pattern to product a raster scan pattern in response to said electrical signal.

69. A method according to claim 68, wherein said step of modifying includes changing the shape of said

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path from a narrow raster scan pattern to a raster scan pattern with greater height.

70. A method according to claim 68, further including the step of determining whether or not said electrical signal exhibits characteristics of a bar code signal, and wherein said step of modifying is performed in response to the results from said step of determining.

71. A method according to claim 70 wherein said step of determining includes deciding whether said bar code symbol is a one dimensional or a two dimensional bar code symbol.

72. A method according to claim 70, wherein said step of determining includes deciding if the scanning direction is substantially orthogonal to the bars of a two dimensional bar code symbol.

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EXHIBIT

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**UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS**

VENTANA MEDICAL SYSTEMS, INC.,

Plaintiff,

v.

VISION BIOSYSTEMS INC.,

Defendant.

Civil Action No. 05-CV-10614-GAO

**VENTANA'S RESPONSES TO VISION'S SECOND SET
OF INTERROGATORIES, NOS. 13 TO 16**

Plaintiff Ventana Medical Systems, Inc. ("Ventana") objects and responds to Defendant Vision BioSystems, Inc.'s Second Set of Interrogatories, Nos. 13 to 16 as follows.

GENERAL OBJECTIONS

Ventana hereby incorporates by reference the General Objections to Ventana's Responses to Vision's First Set of Interrogatories, Nos. 1 to 12, served August 15, 2005.

SPECIFIC OBJECTIONS AND RESPONSES

Ventana expressly incorporates the above general objections as though fully set forth in response to each of the following individual interrogatories. Any response to an interrogatory shall not be deemed a waiver of any applicable specific or general objections to an interrogatory.

INTERROGATORY NO. 13:

If you contend that the '861 patent is valid over the prior art disclosed in Vision's response to Interrogatory No. 15, explain in detail the basis for such belief and identify all persons and documents Ventana relies upon in forming such belief.

RESPONSE TO INTERROGATORY NO. 13:

Ventana objects to this interrogatory as duplicative, overbroad and unduly burdensome because it calls for information that already is in Vision's possession or control in connection with *Vision I*. Discovery on the validity issues was completed in *Vision I*, and the two cases have been consolidated. To the extent Vision's response to Interrogatory 15 attempts to inject new grounds for contesting the validity of the '861 patent that were not raised in *Vision I*, this is improper, and Ventana will not respond to such new grounds unless and until Vision obtains leave of Court to raise such new grounds.

INTERROGATORY NO. 14:

If you contend that the cancellation of claim 72 which specified only the step of "automatically determining whether reagent in the reagent container should be dispensed onto the slide" and the amendment of claim 80 on August 24, 2001 in response to the examiner's rejection so as to require the following limitations: "providing a bar code reader" and "reading a slide bar code placed on the slide using the bar code reader..." was not a narrowing amendment made for reasons of patentability which surrendered the subject matter between the original claim and that of the amended claim for each claim containing the limitation, explain in detail the basis for such belief and identify all persons and documents Ventana relies upon in forming such belief.

RESPONSE TO INTERROGATORY NO. 14:

Ventana objects to this interrogatory as vague and ambiguous because it fails to describe the requested information with reasonable particularity.

Subject to the foregoing objections and its General Objections, Ventana responds on the assumption that Vision is referring to Patent Application No. 09/452309. Ventana states that

contrary to Vision's assertion, dependent application claim 80 was never amended "to require the following limitations: 'providing a bar code reader' and 'reading a slide bar code placed on the slide using the bar code reader...'" Those limitations were always present in dependent application claim 80.

To the extent that Vision believes that dependent application claim 80 was amended by virtue of the fact that it was rewritten in independent form, Ventana states that the claim was not amended for reasons of patentability because the Examiner stated that dependent application claim 80, as originally submitted in the Preliminary Amendment, was allowable if written in independent form.

To the extent Vision contends there is any presumptive surrender of equivalents with respect to the limitations "providing a bar code reader" and "reading a slide bar code placed on the slide using the bar code reader," Ventana states that any such presumption would be inapplicable for at least the following reason. The prosecution history explains the Examiner's reliance on prior art in rejecting application claim 72 (the limitations of which were restated in application claim 80 when rewritten in independent form). Application claim 80 was never subject to a prior art rejection. As this Court held in its September 30, 2004 Memorandum and Order, the prior art cited by the Examiner:

"teaches a system which relies, at least in part, on data entry from the operator. . . . [T]he operator programs that for reagent container position 1, reagent 'A' is selected. Likewise, for slide position 1, histochemical protocol 'z' is selected. The operator must then load reagent container 1 with reagent 'A' and slide position 1 with a slide requiring histochemical protocol 'z'. . . . [T]he computer runs its program under the assumption that the operator placed the reagent containers and the slides in their pre-programmed positions (i.e., the operator has entered 'data' in the form of placing the reagent containers and slides in the proper positions)."

As the Court also held, Ventana distinguished this prior art on the grounds that the claimed invention describes an "automated system" in which the operator "is not required to place the reagent containers or slides in pre-assigned positions." The claimed invention "automatically identifies the reagent container using a computer and automatically determines whether reagent in the reagent container should be dispensed onto the slide. . . . the automatic

identification of the staining protocols and the automatic identification of the reagent containers work in combination to eliminate the need for operator input at the beginning of a staining run.”

This rationale distinguished the claimed invention, which automatically identifies reagents and protocols without operator input, from the prior art systems, which require the manual intervention of an operator to identify the reagents and protocols and to place the slides and reagents into their correct, pre-assigned locations. This rationale bears no more than a tangential relationship to the use of the accused technology in the Vision system, namely OCR. This is so because, unlike the prior art, Vision’s accused system is not a manual system in which the operator must place the reagent containers and the slides in pre-assigned positions. Rather it is an automated system as claimed in the ‘861 patent. Just like slide bar code labels and reagent container bar code labels which allow for the claimed automated functionality described above, Vision’s use of reagent container bar code labels and slide bar code and/or OCR labels are what allow for the same claimed automated functionality. Ventana never disclaimed technologies such as OCR which allow for the same claimed automated functionality.

INTERROGATORY NO. 15:

Explain in detail the basis for your response to Request for Admission No. 1 that “the Bond-OCR uses bar code technology on the slide ID” and identify all persons and documents Ventana relies upon in forming such a belief.

RESPONSE TO INTERROGATORY NO. 15:

Ventana objects to this interrogatory to the extent it calls for information that is protected by the attorney-client privilege and/or the attorney work product doctrine.

Subject to the foregoing objections and its General Objections, Ventana states that as presently advised, the Bond-OCR uses a Slide Labeler to print slide labels that contain a slide ID. The Slide Labeler can be instructed to print slide labels that contain a slide ID either (i) in the form of a 1-D bar code, or (ii) in the form of an optical character code. The instructions for doing so are set forth in several technical documents produced by Vision, including without limitation, the Service Manual for the Bond-OCR. The Bond-OCR also uses the Jada FM-204

to read the slide labels placed on slides. The same Jadak scanner is used to read slide IDs in the form of a 1-D bar code as well as to read slide IDs in the form of an optical character code.

Vision has produced several technical documents that describe this operation, including without limitation, the Service Manual for the Bond-OCR. Vision employees are knowledgeable about the foregoing functionality of the Bond-OCR, including without limitation, Ross Barrow and Paul Sorenson.

INTERROGATORY NO. 16:

Identify each witness you intend to call at trial and for each witness, state each fact and/or opinion about which you expect the witness to testify, and for each fact and/or opinion, identify each document or thing which you contend supports the fact or opinion.

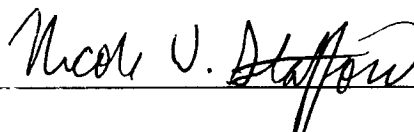
RESPONSE TO INTERROGATORY NO. 16:

Ventana objects to this interrogatory as premature. As presently advised, Ventana has not yet determined the identity of the witnesses it will call at trial. Ventana will provide such information consistent with the Court's pre-trial procedures. Ventana objects to disclosing the substance of any expected testimony to be provided by fact witnesses on the grounds that such information is protected by the attorney client privilege and/or work product immunity. Ventana will disclose expert opinions and the bases therefore as required by Rule 26, F.R.Civ.P. in accordance with the schedule agreed to, or to be agreed to, by the parties.

Dated: September 19, 2005

VENTANA MEDICAL SYSTEMS, INC.

By its attorneys,

A handwritten signature in black ink, appearing to read "Nicole W. Stafford", is written over a horizontal line.

Nicole W. Stafford (*pro hac vice*)
WILSON SONSINI GOODRICH & ROSATI
Professional Corporation
8911 Capital of Texas Highway North
Westech 360, Suite 3350
Austin, Texas 78759-8497
Telephone: (512) 338-5400

Ron E. Shulman (*pro hac vice*)
Roger J. Chin (*pro hac vice*)
WILSON SONSINI GOODRICH & ROSATI
Professional Corporation
650 Page Mill Road
Palo Alto, California 94304
(650) 493-9300

Brian L. Michaelis (BBO #555159)
BROWN RUDNICK BERLACK ISRAELS, LLP
One Financial Center
Boston, Massachusetts 02111
(617) 856-8200

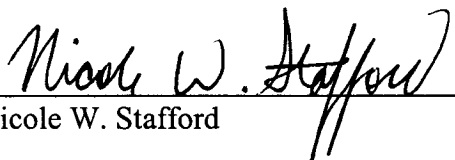
Jeffrey N. Danis (BBO #113880)
VENTANA MEDICAL SYSTEMS, INC.
1910 Innovation Park Drive
Tucson, Arizona 85737
(520) 229-3965

CERTIFICATE OF SERVICE

I hereby certify that a true and correct copy of the foregoing pleading was served, via facsimile and overnight courier, on counsel for defendants in this matter on this 19th day of September, 2005.

Elizabeth A. Leff, Esq.
Rothwell, Figg, Ernst & Manbeck, P.C.
1425 K Street, NW, Suite 800
Washington, DC 20005

Christine M. Roach, Esq.
Roach & Carpenter, PC
24 School Street
Boston, MA 02108



Nicole W. Stafford

EXHIBIT

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US005646046A

United States Patent [19]**Fischer et al.**[11] **Patent Number:** **5,646,046**[45] **Date of Patent:** **Jul. 8, 1997**

[54] **METHOD AND INSTRUMENT FOR
AUTOMATICALLY PERFORMING
ANALYSIS RELATING TO THROMBOSIS
AND HEMOSTASIS**

[75] **Inventors:** **Timothy J. Fischer**, Raleigh; **Janet B. Callahan**, Chapel Hill; **Paul Joseph Braun**, Durham; **Thomas Beecher Givens**, Rougemont, all of N.C.; **Julie F. Hoffman**, Ypsilanti, Mich.; **William Chester Hulette**, Hillsborough, N.C.; **John Glenn Link**, Durham, N.C.; **Charles Hermas Swope**, Raleigh, N.C.

[73] **Assignee:** **AKZO Nobel N.V.**, Arnhem, Netherlands

[21] **Appl. No.:** **389,986**

[22] **Filed:** **Feb. 14, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 107,381, Aug. 16, 1993, abandoned, which is a continuation-in-part of Ser. No. 833,950, Feb. 11, 1992, Pat. No. 5,236,666, which is a continuation-in-part of Ser. No. 443,951, Dec. 1, 1989, abandoned.

[51] **Int. Cl.⁶** **G01N 35/02**

[52] **U.S. Cl.** **436/49; 436/43; 436/47; 436/48; 436/50; 436/55; 422/63; 422/65; 422/67; 422/73**

[58] **Field of Search** **422/63-67, 68.1, 422/73, 82.05, 105; 436/43, 47-50, 54, 55, 174, 180, 164, 807; 364/497**

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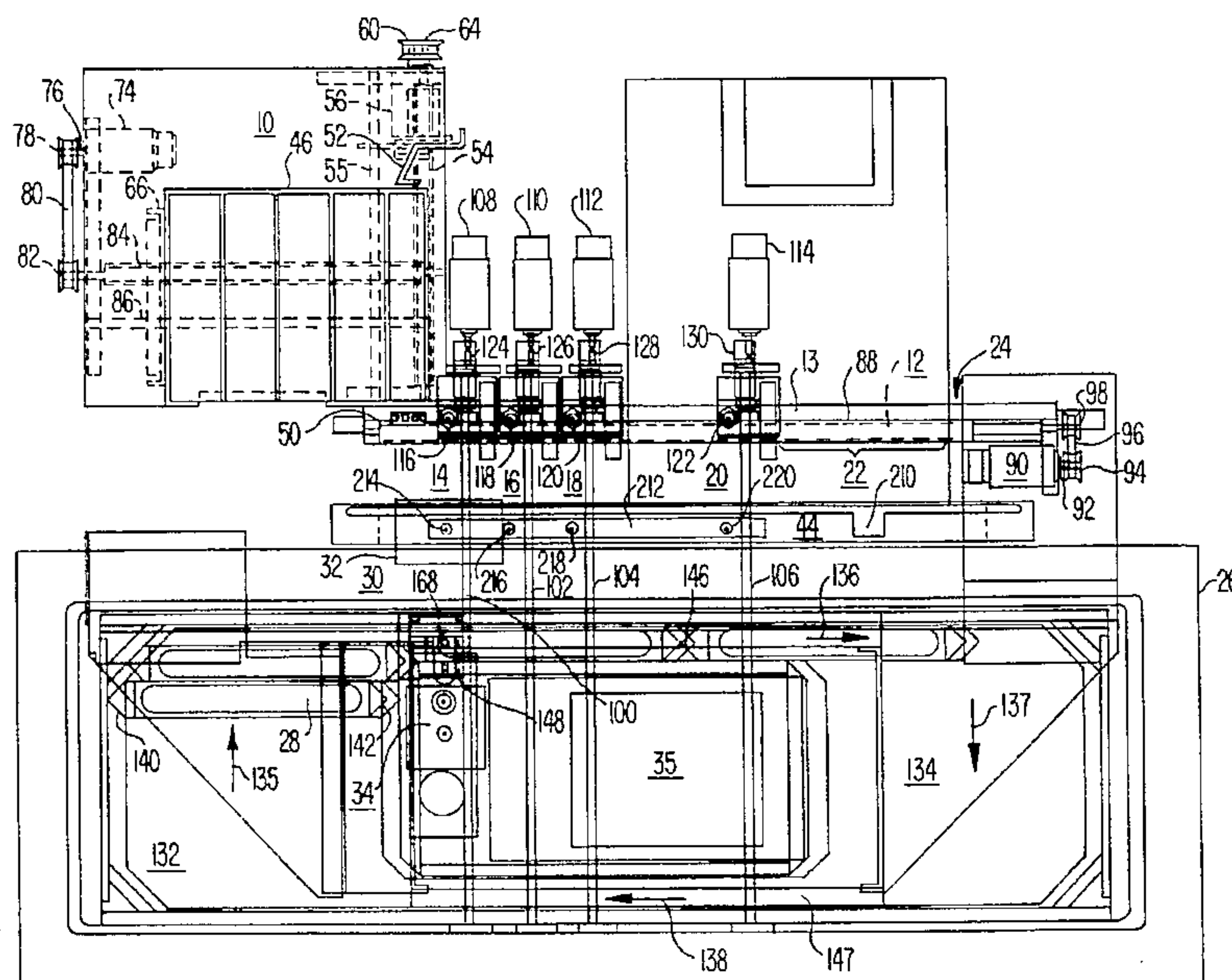
Primary Examiner—Long V. Le

Attorney, Agent, or Firm—Gregory R. Muir; William M. Blackstone

[57] ABSTRACT

This invention relates to a novel, fully automated spectrophotometric analyzer and method used for testing blood samples in the clinical laboratory for thrombosis and hemostasis properties. The analyzer tests samples in a fully randomized format, and is fully automated in the areas of specimen handling, sample preparation, optical inspection, data analysis and total quality control for imprecision and bias.

40 Claims, 10 Drawing Sheets



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Page 2

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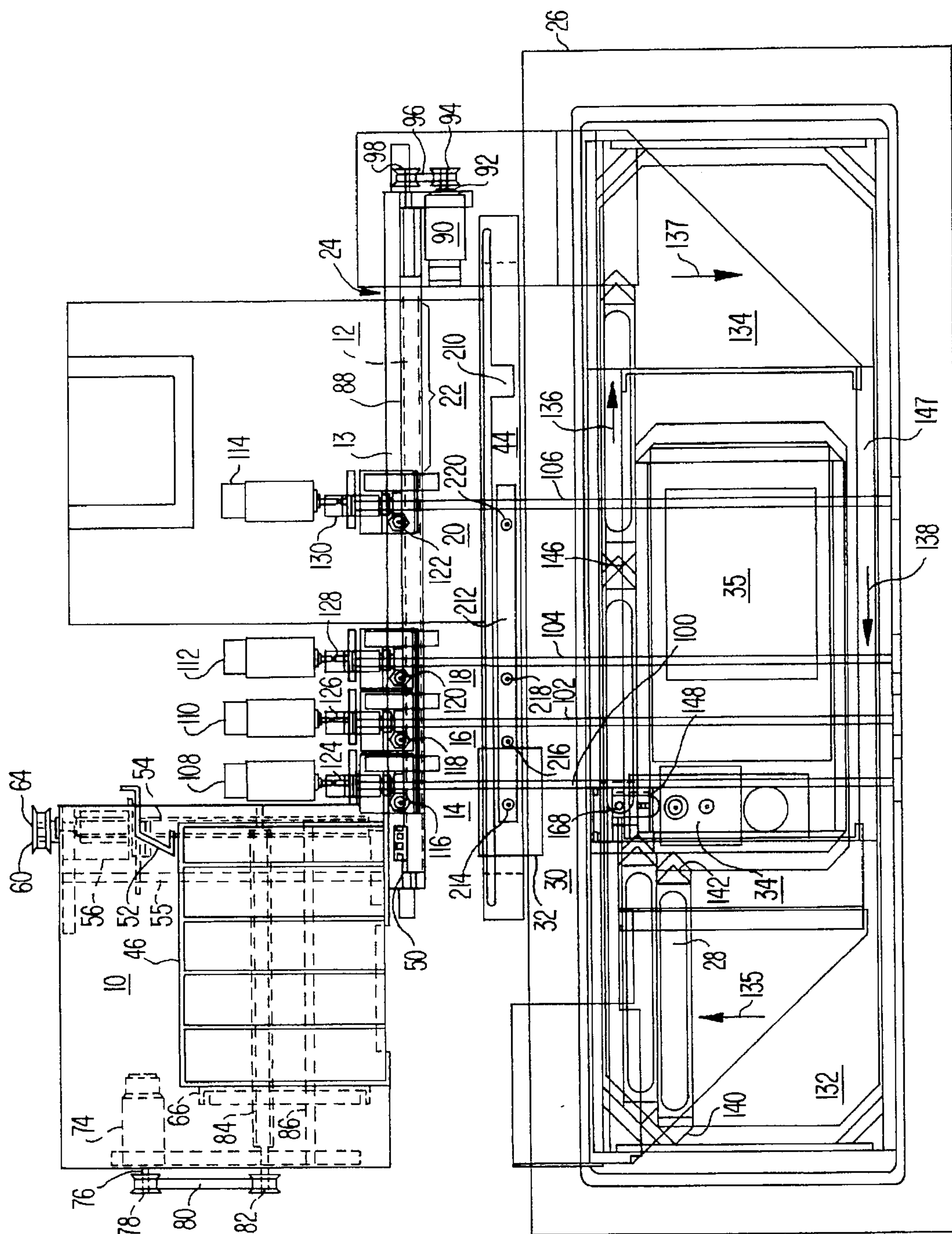
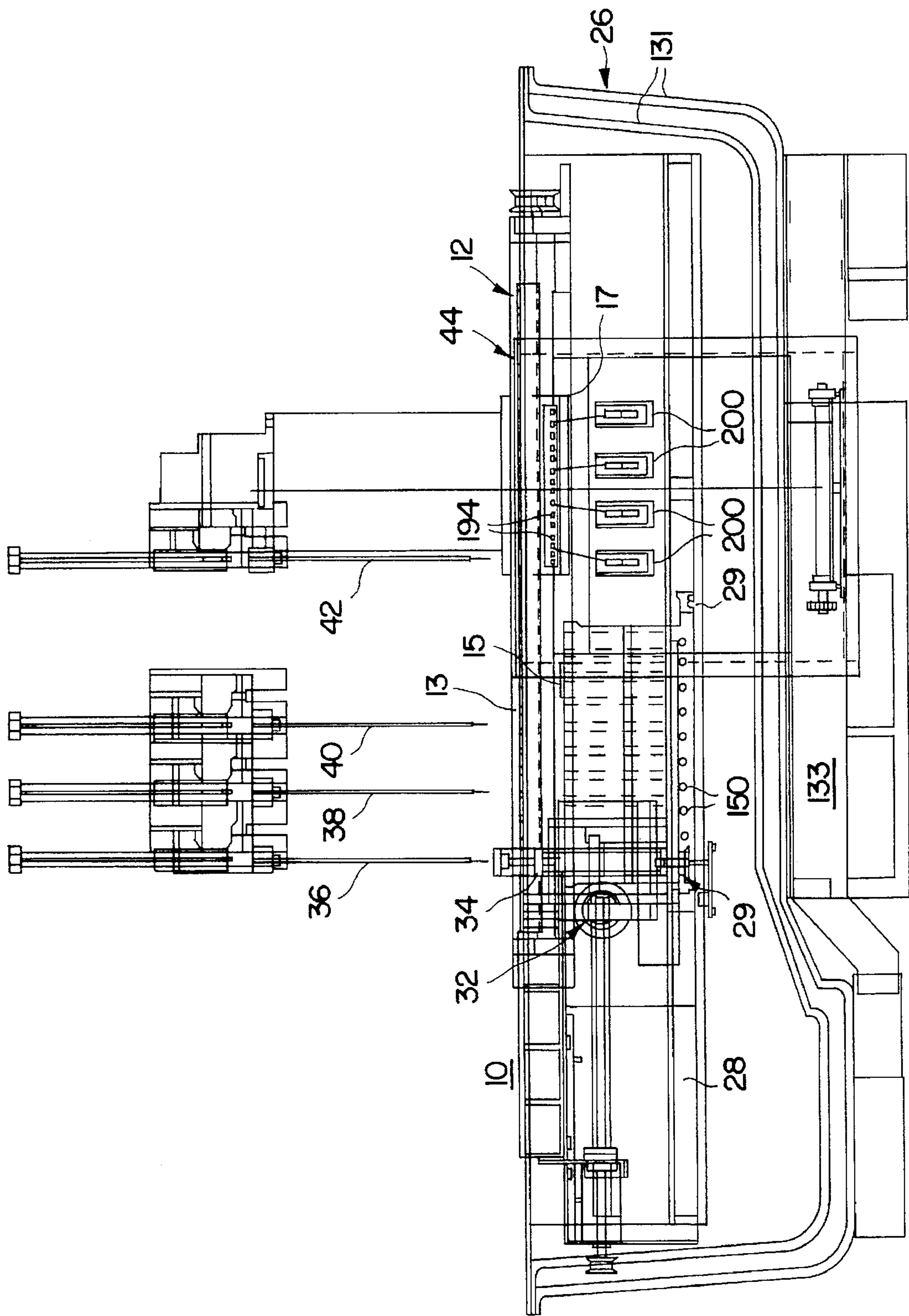


FIG. 1





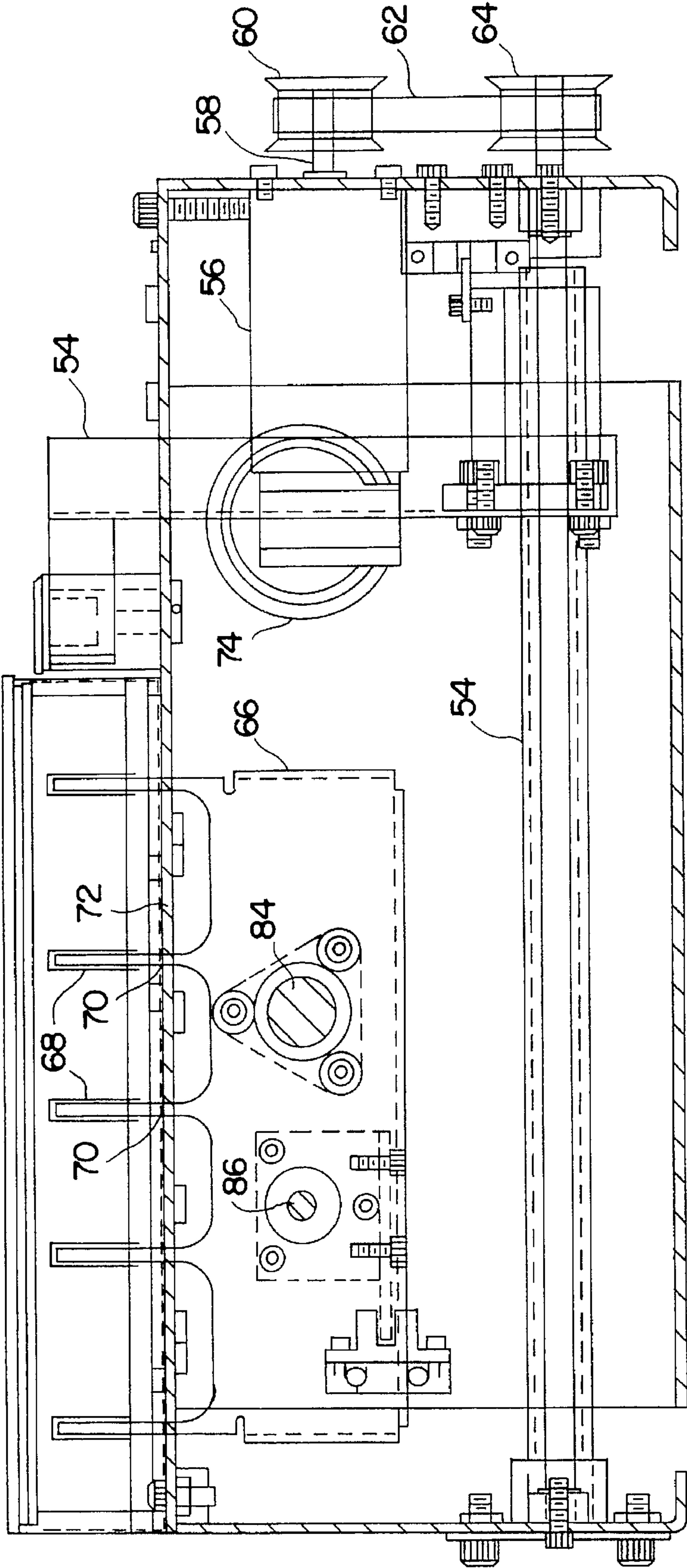


FIG. 4

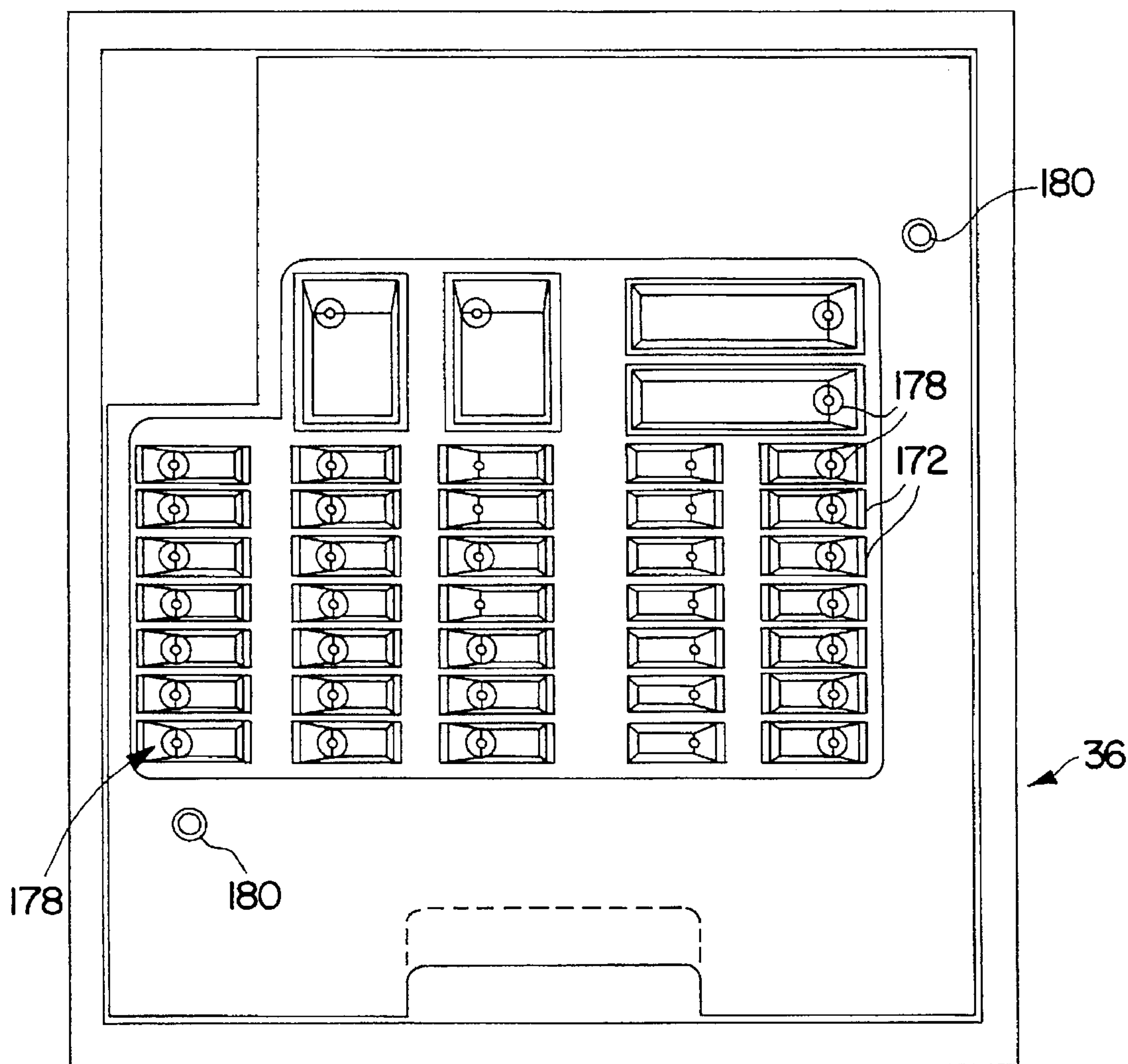


FIG. 5

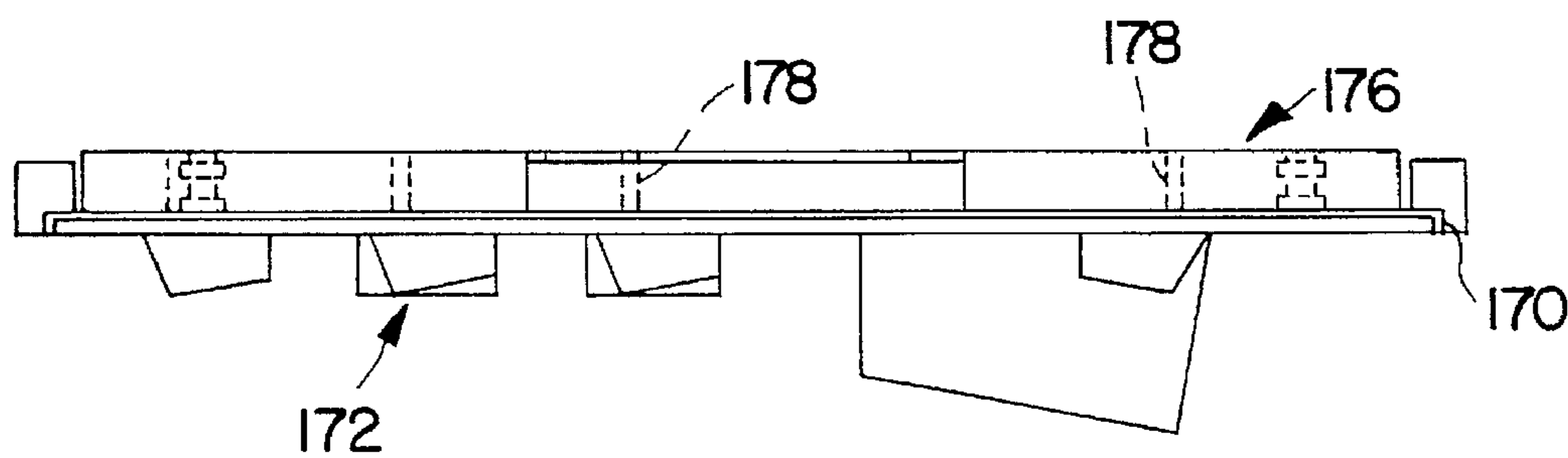
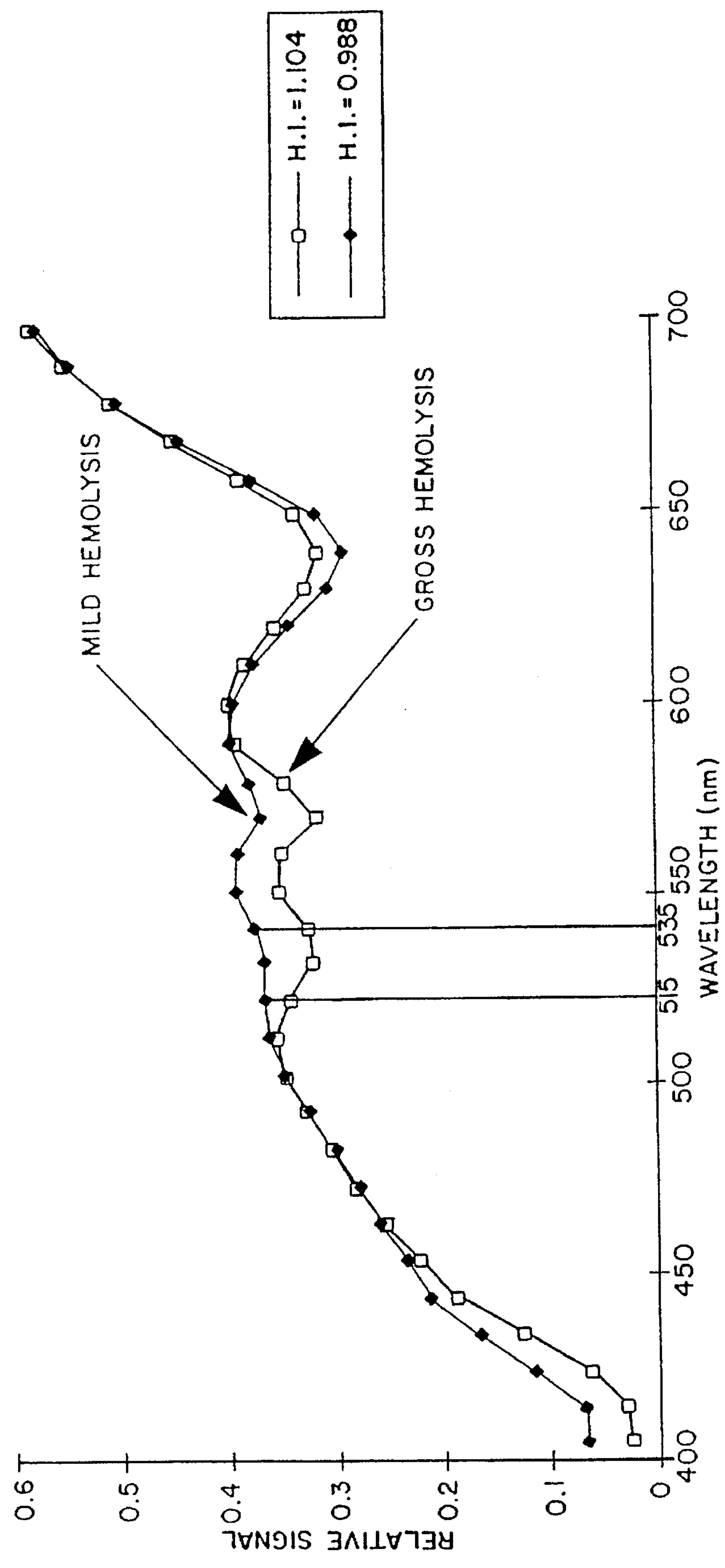


FIG. 6

FIG. 7



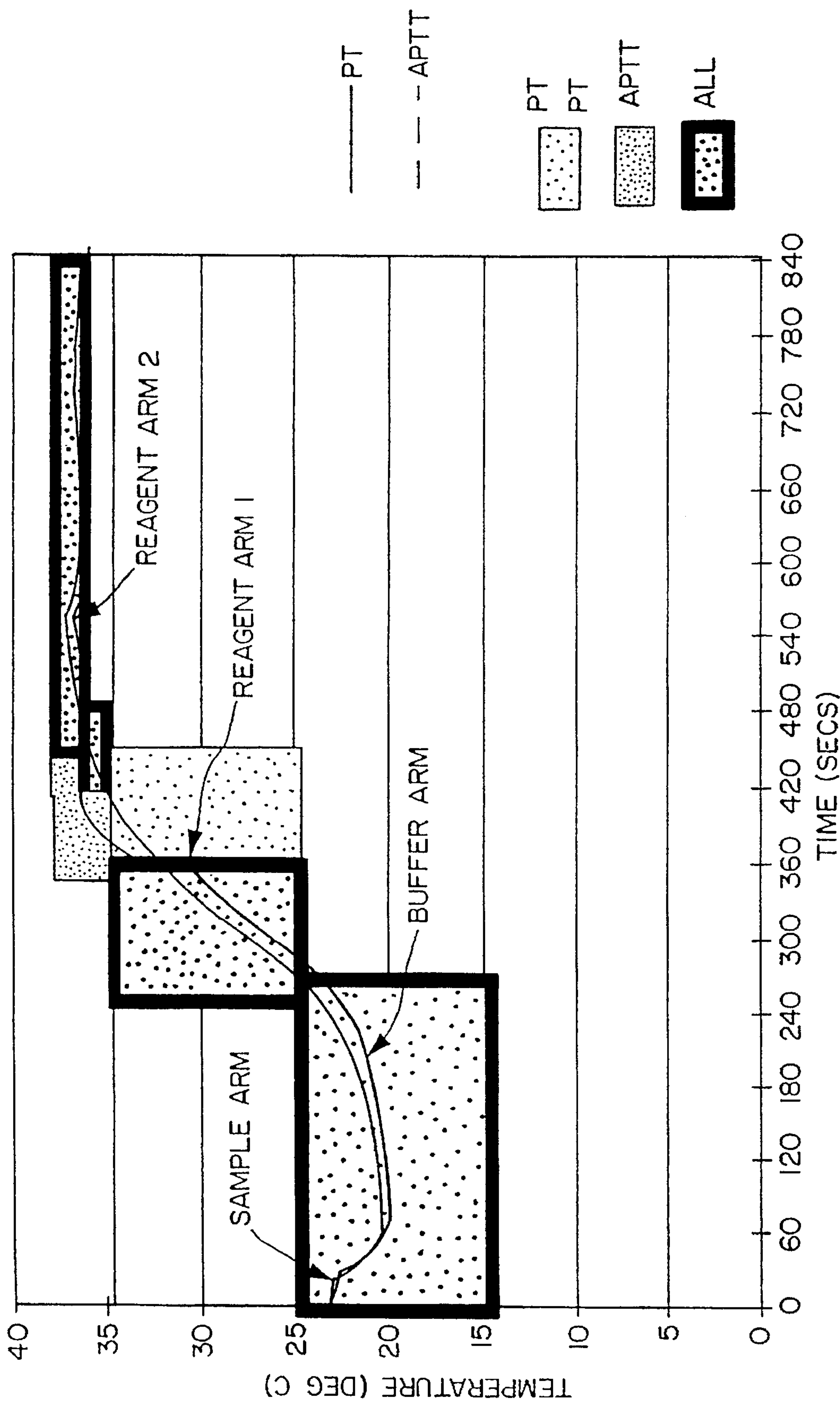


FIG.8

FIG. 9

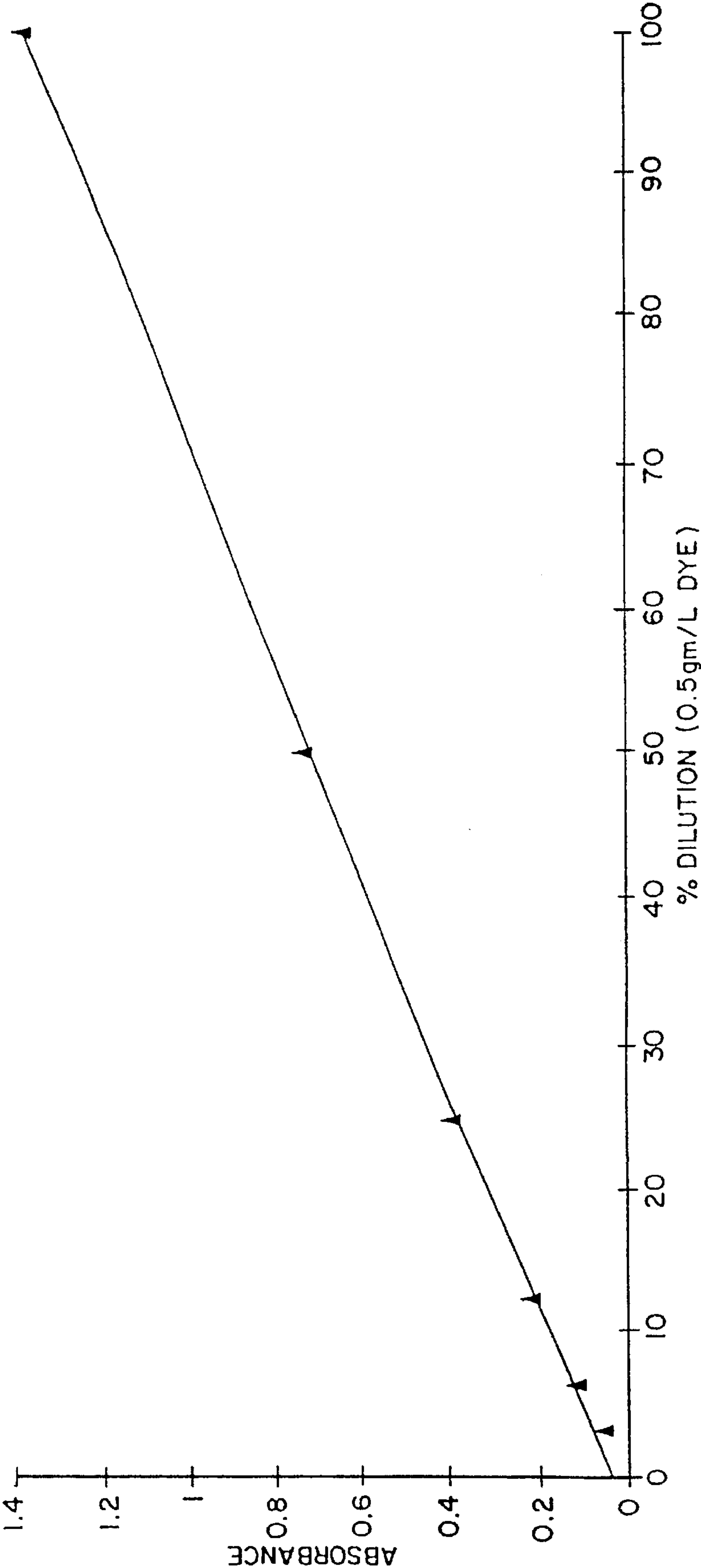


FIG. 10

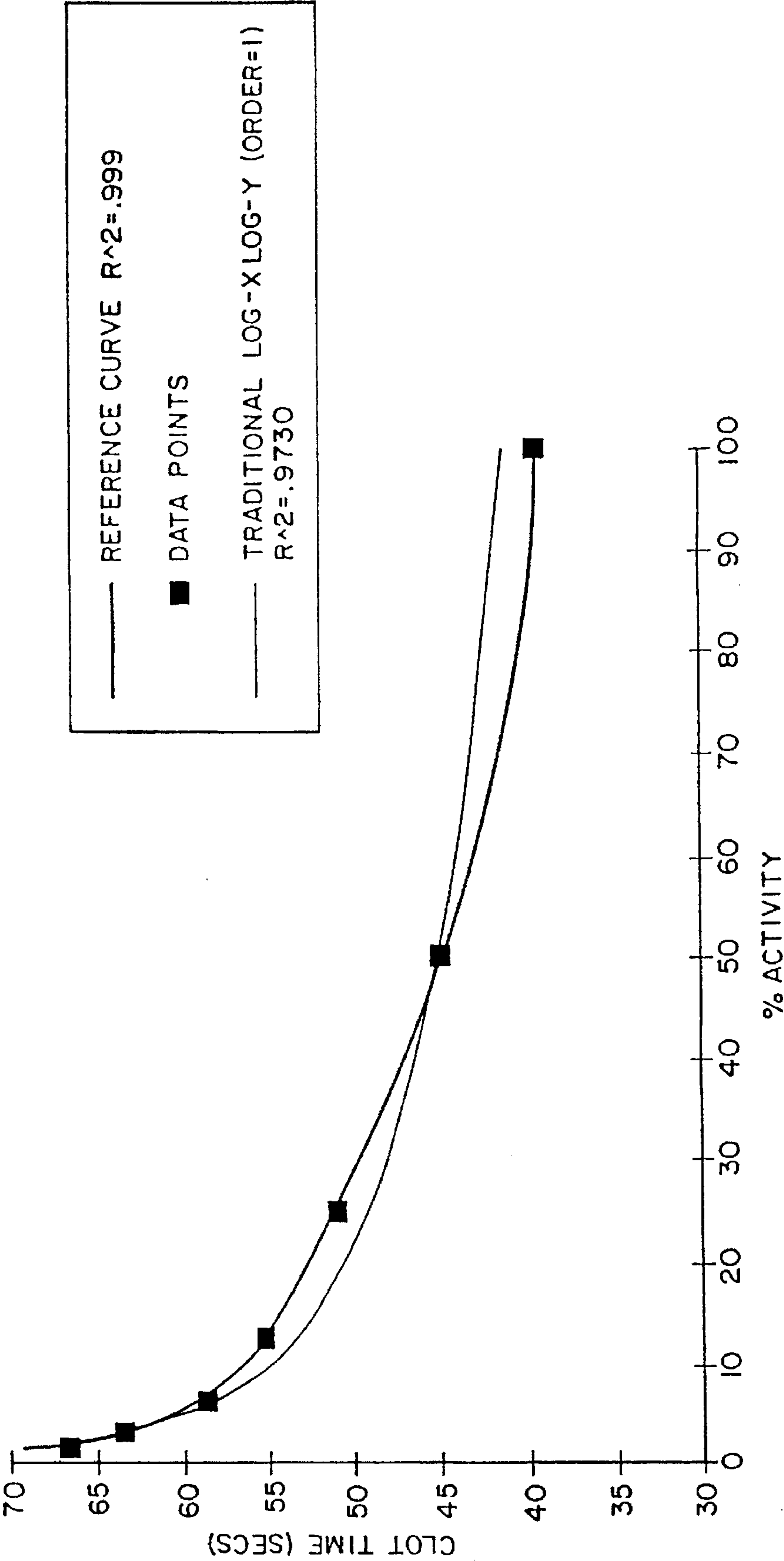
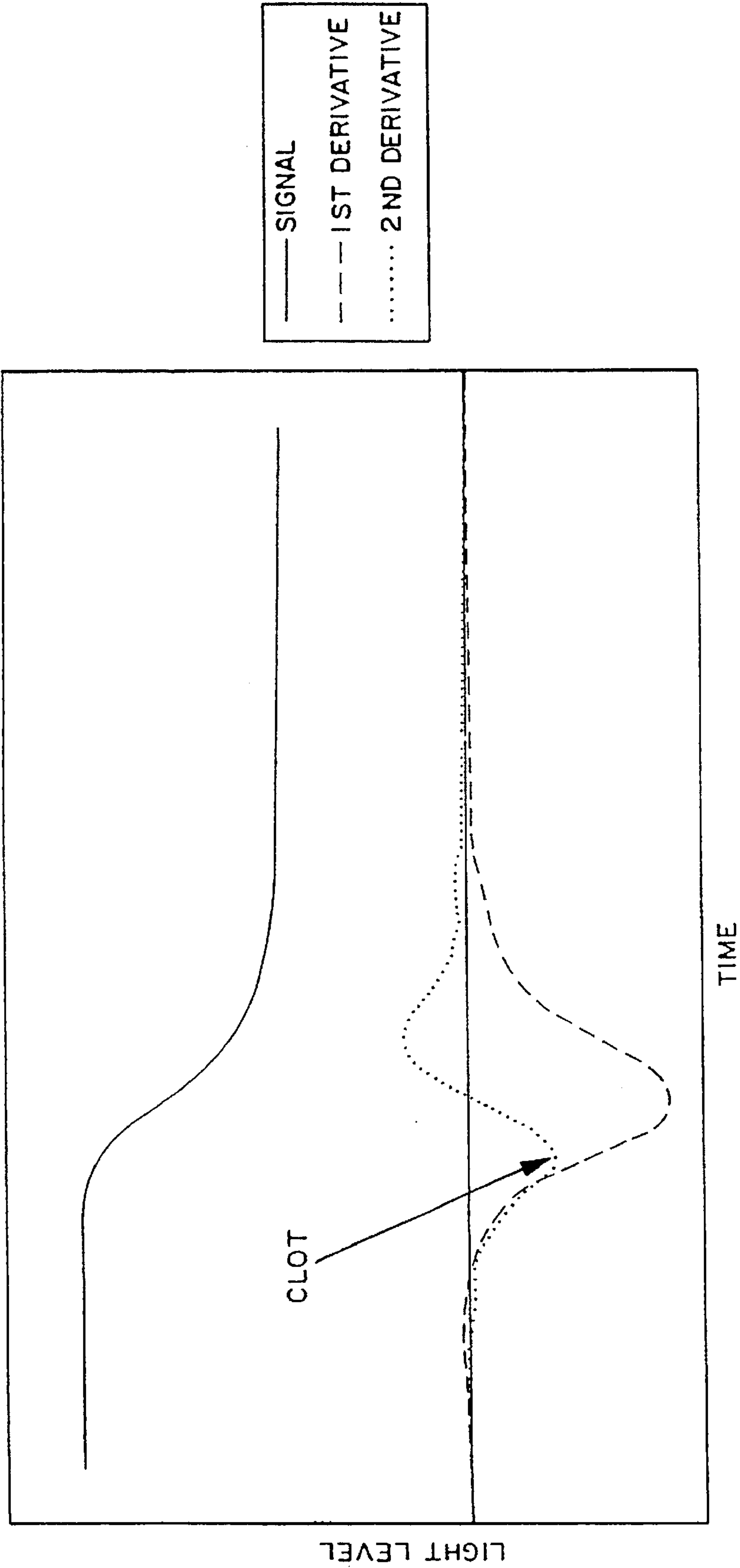


FIG. 11



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METHOD AND INSTRUMENT FOR AUTOMATICALLY PERFORMING ANALYSIS RELATING TO THROMBOSIS AND HEMOSTASIS

This is a continuation of U.S. Ser. No. 08/107,381, filed Aug. 16, 1993, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07/833,950, filed Feb. 11, 1992 now U.S. Pat. No. 5,236,666 which is a continuation-in-part of U.S. Ser. No. 07/443,951, filed Dec. 1, 1989, now abandoned.

This application is additionally related to the following copending U.S. Patent Applications and issued patents, which are owned by the Assignee of the present Application, and the disclosures of all but No. 6 are incorporated herein by reference:

(1) Ser. No. 07/443,952, to Swope et al., titled "Multi-channel Optical Monitoring Systems", now U.S. Pat. No. 5,002,392;

(2) Ser. No. 07/443,956, to Karp et al., titled "Cuvette and Linear Drive Mechanism Therefor", now U.S. Pat. No. 5,040,894; and

(3) Ser. No. 07/443,954, to Hoffman et al., titled "Apparatus and Method for Cleaning Reagent Delivery Probes", now U.S. Pat. No. 4,989,623.

(4) Ser. No. 07/443,784, to Karp et al., titled, "Cuvette", now U.S. Patent Des. 325,090.

(5) Ser. No. 07/674,957, to Keiter et al., titled, "Heated Liquid Sampling Probe for an Automated Sampling Apparatus," now U.S. Pat. No. 5,178,019.

(6) Ser. No. 07/916,712, to Lewis et al., titled, "Cassette and Cuvette Loading Mechanism", now U.S. Pat. No. 5,364,592.

(7) Ser. No. 07/896,579, to Haugen, titled, "Method for Scanning Photodiodes", now U.S. Pat. No. 5,245,176.

(8) Ser. No. 07/443,953, to Driscoll, titled, "Method of Monitoring Reagent Delivery in a Scanning Spectrophotometer," now U.S. Pat. No. 5,068,181.

This invention relates to a novel, fully automated spectrophotometric analyzer and the method used for assaying for thrombosis and hemostasis properties of blood samples. The analyzer tests samples in a fully randomized format, and is fully automated in the areas of specimen handling, sample preparation, optical inspection, signal processing and quality assurance/control for imprecision and bias allowing for numerous assays to be performed on a single sample. The assays vary considerably in nature and fall into three categories: clot based, chromogenic, and immunological. No single automated methodology or instrumentation currently exists that successfully performs all of these types of assays concurrently.

BACKGROUND OF THE INVENTION

Thrombosis and hemostasis testing is the in vitro study of the ability of the blood to form clots and to break clots in vivo. As thrombotic and hemostasis pathways form a part of very important disease states ranging from hemophilia to strokes and heart attacks, the testing of a patients capabilities in thrombosis and hemostasis is a critical diagnostic tool. Should a patients ability to form clots in vitro fall outside of the established norm, or should certain markers be out of the normal range, the serum or plasma sample is further assayed to determine the reason for the problem. These assays are in standard use in all hospital laboratories.

Coagulation assays began, and are still done in many instances, in a test tube using hand methods. Early on, the goal was to determine if a patients blood sample would clot

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after certain materials were added. It was later determined that the amount of time it took for the sample to clot was related to congenital or acquired disorders. This type of testing is extremely dependent on the laboratory technologist, and so, some form of standardization was seen to be needed. As technology improved and stronger correlations between in vivo conditions and in vitro assays were established, semi-automatic coagulation analyzers began to appear.

These coagulation analyzers primary usefulness is to remove the subjectiveness in determining the exact second a clot forms in a sample in vitro. However, these analyzers did and do not have the automation required to remove variability associated with sample preparation. Furthermore, advances in clinical thrombosis and hemostasis assays resulted in the development of new types of assays that aided in the diagnosis and treatment of a patient and semi-automated coagulation analyzers seldom possess the ability to perform more than one assay at a time. This is because reagent pathways are dedicated to a single reagent, resulting in a limited number of assays that can be performed on each instrument. Generally, the semi-automated instrumentation performs one test in a batch mode, maintaining one profile of temperature vs. time for each different type of assay.

Semi-automated analyzers also require the technician to manually deliver the plasma sample. A new sampling device, generally a pipette tip, is used for each specimen to eliminate plasma cross-contamination between samples. Using a common sampling means for reagents and samples requires novel approaches to eliminate cross contamination of samples and reagents.

In order to have the next generation of analyzers, fully automated analyzers must be developed to be able to use the same sampling device for all specimens and to have common pathways for delivery of multiple reagents, and to provide a universal time and temperature profile compatible with a multitude of assays.

Additionally, any complex computations needed to be performed for an assay are done by an operator when semi-automated analyzers are used. Differences in operators techniques in analyzing data lead to increased levels of inaccuracy of the data. Another feature needed to improve coagulation testing is improved and standardized data analysis techniques to obtain the desired performance characteristics from inter and intra laboratory comparisons, which would result in a higher standard of care for the patient.

Quality control and system monitoring of the semi-automated coagulation analyzers are primitive and inadequate when compared to the state of the art.

The next generation of analyzers, a fully automated thrombosis and hemostasis analyzer, requires a statistically controlled, on-line quality assurance program that monitors the system integrity, as the analysis are being performed. This program must not only identify failures after they have occurred, but predict potential failures before they occur.

Another area of the clinical laboratory, the clinical chemistry laboratory, has had fully automated analyzers for a number of years. The tests performed and the types of reactions read, including colorimetric, fluorescent and luminescent measurement, are substantially different and have endpoints that are easier to detect than do coagulation-based tests, those performed in the coagulation laboratory. The same progress towards full automation has not been seen in the coagulation laboratory as in the clinical chemistry laboratory.

In general, the basic tests or assays performed in the coagulation laboratory using plasma, serum or whole blood

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include performing the Partial Thromboplastin Time ("PTT"), the Prothrombin Time ("PT"), the Activated Partial Thromboplastin Time ("APTT"), testing for deficiencies in Factors such as Factors II, V, VII, VIII, IX, XI, XII and others, and chromogenic and immunological testing for thrombosis or hemostasis markers. These, among others, have proven to be much more difficult to do on automated equipment than have the clinical chemistry tests. This is because the tests run in the coagulation laboratory usually: (1) involve unique time/temperature profiles; (2) are extremely sensitive to both reagent and plasma carryover; (3) require unique data analysis; and (4) have unique quality control requirements.

A method for automatically performing a variety of coagulation-related assays and a fully automated coagulation analyzer is needed to perform a host of assays in a totally random format that would expedite patient diagnosis. It must have the ability to control the sample preparation stages of an in vitro assay; to perform all thrombosis and hemostasis assays using a common temperature profile; to measure the reaction that occurs when the appropriate materials are added; and to determine both the immediate response as well as to provide mathematical tools for calculating complex results. This entire process should be monitored using an on-line quality control package that is designed to minimize imprecision associated with random error and to minimize bias associated with error due to the system itself, systemic error.

This type of fully automated coagulation analyzer would provide more accurate results that in turn would allow for quicker and more accurate diagnosis of current or predicted illness, thereby allowing for better treatment of the patient.

SUMMARY OF THE INVENTION

The present invention includes a method for automatically assaying for hemostasis and thrombosis parameters in a plasma, serum or whole blood sample comprising:

- a) providing a programming input means for identifying the sample and scheduling one or more hemostasis and thrombosis-related assays to be performed on the sample;
- b) providing a specimen handling means for automatically transferring an aliquot of the sample from a holding device to a test well in a cuvette;
- c) providing a sample preparation means for automatically adding reagents needed for an assay to measure hemostasis or thrombosis parameters to the sample in the test well at a specified time and temperature, accommodating a universal thermal profile, to obtain a reaction, wherein the order of the reagents added can be in a random access format thereby eliminating the need for batch analysis;
- d) providing a detecting means for detecting the reaction in the well and measuring the data from the reaction;
- e) providing a processing means for mathematically processing the measured data to evaluate a change in or magnitude of the measured data from the reaction in the well;
- f) providing a reporting means to report said results of the evaluation by the processing means; and
- g) simultaneously with all of steps a)–f) above, providing a quality assurance means for monitoring the performance of the method and evaluating the validity of the reported data for the sample,

thereby automatically performing hemostasis and thrombosis-related assays and determining and reporting results on hemostasis and thrombosis parameters in the sample.

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The present invention also provides a fully automated coagulation analyzer that responds to all of the needs stated above. The linear, spectrophotometric analyzer performs multiple hemostasis and thrombosis assays on samples of serum, plasma or whole blood in a totally random order. It is fully automated in terms of specimen handling, sample preparation, optical inspection, signal processing, and total quality control for imprecision and bias. All hemostasis and thrombosis assays performed on the analyzer have been designed to share a common temperature profile, thereby allowing the random performance of assays. Each of these functions are also internally controlled through quality assurance programming. Each assay is defined by an assay definition file ("adf") allowing for flexibility in method definition. This flexibility is required in order to obtain the unique performance characteristics for each assay. Improvements in linearity, accuracy and the minimization of bias can be achieved by optimization of adf parameters.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic top elevation of a sample handling system in a optical evaluation instrument according to the invention.

FIG. 2 is a schematic front elevation of FIG. 1.

FIG. 3 is a schematic right-side elevation of FIG. 1.

FIG. 4 is a schematic right-side elevation of the cuvette storage device of FIG. 1.

FIG. 5 is a top elevation of the reagent container block of FIG. 1.

FIG. 6 is a side elevation of FIG. 5.

FIG. 7—The relative signal at various wavelengths is shown for two samples: a sample with mild Hemolysis, and a sample with Gross Hemolysis. Wavelengths of 515 nm and 535 nm are identified. The actual data collected is represented by data markers and these data markers are connected for each sample.

FIG. 8—The temperature of a sample is shown for a PT assay definition and an APTT assay definition as a function of time. Each sample/reagent delivery location is identified. The acceptable temperature ranges at each instant in the temperature profile are identified by "zones."

FIG. 9 shows the relative absorbance rates of a dye sample, serially diluted. A Least Squares regression line is also shown, and its goodness-of-fit measure r^2 .

FIG. 10 shows the actual data points representing the clot times recovered for a serially diluted reference, and (1) the traditional curve and (2) the new curve fit through those points.

FIG. 11 shows a smoothed signal, smoothed first derivative of that signal, and smoothed second derivative for data collected from a normal APTT assay. The location of the maximum of the second derivative (the clot time), is identified.

DETAILED DESCRIPTION OF THE INVENTION

The needs of the clinical coagulation laboratory for a fully automated coagulation analyzer have been met with this invention. Provided is an optical evaluation instrument and method that can handle a high throughput of patient samples with a high degree of versatility, adaptability and reliable automation. This is walkaway automation once patient samples still sealed in the original evacuated collection tube are loaded into the system.

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The present invention includes a method for automatically assaying for hemostasis and thrombosis parameters as described above, in a plasma, serum or whole blood sample in a holding device, such as an evacuated collection tube, that can be stoppered. The assays may be performed in either a batch or random fashion, wherein the same assay or test is performed on all samples, multiple different assays are performed on each sample and a different assay is performed on each sample. This is accomplished by providing an integrated programming input means for identifying the sample and scheduling one or more hemostasis and thrombosis-related assays to be performed on the sample in any order. The programming means will accept and will allow the assays to be performed in a random format, i.e. in any order.

Next in the method is providing a specimen handling means for automatically transferring an aliquot of the sample from a holding device or a collection tube, with or without a stopper, to a test well in a cuvette. Because of various issues regarding the exposure of laboratory workers to blood borne contagious disease, being able to provide a specimen handling means to piece the septum or stopper without a human present is a definite asset in a clinical instrument or method.

Also, in the method is providing a sample preparation means for automatically adding the reagents needed for an assay to measure hemostasis or thrombosis parameters to the sample in the test well at a specified time and temperature, accommodating a universal thermal profile, to obtain a reaction, and wherein the order of the reagents added can be in a random format eliminating the need for batch analysis. This methodology is needed because each assay or test performed in the coagulation laboratory is done with different time and temperature needs. The present method provides for a common universal thermal profile wherein each assay or test can be done within its particular parameters. The method provides for a heating and cooling track where each sample moves forward, but each at the speed needed for the assay to be performed, and the means for automatically adding the reagent needed at the proper temperature. Once the proper reagents are added at the proper time and temperature, the reaction begins.

Next, the method provides a detecting means for detecting the reaction in the well, and takes the result of said detection to mathematically compute data. This data, or the raw result of the assay or test, is provided a processing means wherein the computed data is evaluated to determine the change in or the magnitude of the reaction in the well, and when required, to transform the data into a diagnostically useful result.

Simultaneously with all of steps above, the method provides a quality assurance means for monitoring the performance of the method and evaluating the validity of the reported data for the sample, thereby automatically performing hemostasis and thrombosis-related assays and determining and reporting results on hemostasis and thrombosis parameters in the sample.

A preferred way of performing the above described method of testing a large number of samples in a random order is done with the following instrument.

Overview of the Automated Analyzer

The analyzer is fully automated in terms of specimen handling, sample preparation, optical inspection, signal processing and total quality control for imprecision and bias, and a quality assurance program.

A. The Specimen Handling Segment

The specimen handling segment of the analyzer is divided into four basic components consisting of positive patient

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identification of the sample, screening for preanalytical variables, the ability to do closed container sampling and the ability to continuously supply cuvette wells for sample evaluation.

In greater detail, the specimen handling segment consists of:

1) a means for storing and continuously supplying a plurality of cuvettes to be used in the assays, each cuvette containing a plurality of reaction wells;

2) the optically readable code, such as a bar code or other coding, present on or incorporated into the sample collection tube or holding device containing a sample of serum, plasma or whole blood, is a patient identification and tracking device, and is also used in assay entry and automatic tracking of the assay during sample evaluation;

3) a means for the screening of and evaluating preanalytical variables prior to the beginning of any assay, such as hemolysis, bilirubin and lipemia; and

4) a sample insertion station including a means for automatically aspirating sample from the sample collection tube or holding device with or without a stopper and for automatically dispensing the aspirated sample into a reaction well of a cuvette.

B. The Sample Preparation Segment

The sample preparation segment of the analyzer consists of four components: the means for defining unique reagent transferring sequences for each assay, random access ability, or the ability for a probe to aspirate reagent from a reagent container and dispense it into a predetermined cuvette well in any order and to aspirate and deliver different plasmas without cross contamination; the universal profile testing method; a means for performing auto dilutions of the sample; and a means for monitoring reagents and samples.

In greater detail, the sample preparation segment consists of:

1) an assay definition file ("adf") that allows for flexibility in how the reagents and plasmas are delivered. This flexibility is defined in terms of aspiration and dispense velocities, temperature, time out (delay) or timing sequences;

2) a reagent station, including a sample preparation means having the ability to randomly aspirate selected amounts of selected reagents from selected reagent containers as needed, and for dispensing the aspirated reagents into a reaction well of a cuvette according to the directions given in a programmed test for the sample in that reaction well. Each well of the cuvette may be programmed to have a different assay performed, and the reagent and sample in the reaction well forms a reaction volume which exhibits optical characteristics to be monitored by the analyzer;

3) a means for providing a universal temperature profile for the different assays programmed on the analyzer, which is an arrangement for temperature regulation of a fluid sample in a cuvette transported through various stations of the automated system for optically monitoring the sample in the cuvette, comprising: a means for transporting the cuvette through the various stations of the sample and reagent delivery system and optical monitoring system, the sample temperature being controlled by the system; cooling means for cooling the sample in the first portion of the profile; heating means for heating the sample in the third portion of the profile in such a way as to maintain the sample temperature within base tolerance constraints; and a second section providing a means of providing a temperature ramp that defines the sample transition from the initial cool temperature to final warm temperature;

4) a means for automatically diluting samples, reference materials, and control materials through the use of pro-

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grammed probes that perform a wide range of serial and nonserial dilutions; and

5) a temperature controlled housing for storing a plurality of reagent containers, each containing a respective reagent, and a plurality of sample collection tubes, each containing a fluid sample and presenting an optically readable code, such as a bar code or other equivalent coding, identifying the sample and a test to be performed on the sample. There is also a reagent tracking system that monitors the amount of available fluid in each discrete vial or container of reagent, present as a means for sensing liquid levels, one embodiment being a liquid-level sensor on each probe that aspirates and dispenses liquid.

C. The Optical Inspection Segment

The optical inspection segment of the automated analyzer consists of three components, 1) a means for multiple wavelength analysis; 2) the use of a broad spectrum of wavelengths; and 3) continuous normalization of the fluctuations in light levels associated with sample to sample variability.

The optical inspection portion of the analyzer is provided for by:

1) and 2) a multichannel optical monitoring system as described in commonly owned U.S. Pat. No. 5,002,392, issued on Mar. 26, 1991, and incorporated herein by reference and commonly owned U.S. patent application Ser. No. 07/896,579, "Method for Scanning Photodiodes", also incorporated herein by reference; and

3) fluctuations in light levels associated with sample to sample variability, such as differences in color from sample to sample, are normalized through a quality assurance program prior to data analysis of the test results.

D. The Signal Processing Segment

The signal processing segment of the analyzer consists of three components: the determination of kinetic endpoints; complex processing that determines endpoints other than clot formation, such as immunological complexes or chromogenic endpoints; and an on-line database against which each test result can be compared.

In greater detail, the signal processing segment of the analyzer consists of:

1) a means for determining kinetic endpoints, for example, a computer program, that is able to determine the rate of acceleration that a clot is forming. This analysis is unique in that it is based on the kinetics of clot formation as opposed to the use of a threshold, which is sensitive to biological, mechanical and electrical artifacts, thereby giving more accurate test results.

2) a means for determining the endpoint of a variety of assays other than those based on clot formation. For example, chromogenic assays such as ATIII and Protein C, and immunologic assays based on the interactions of antigens and antibodies, such as the assay for D-dimer, are read based on color changes or the presence or absence of agglutination or some type of label; and

3) a means for creating and storing reference curves that can be used for the evaluation of controls and patient samples.

Each of the above segments of the coagulation analyzer is integrated with on-line quality control and quality assurance programming. These are conducted to minimize bias and imprecision throughout the analyzer.

E. The Quality Assurance Segment

The satisfactory performance of any and all clinical laboratory assays depends on an effective quality control or quality assurance program, which controls each of the parameters listed below. Control of these parameters mini-

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mizes imprecision associated with random error and minimizes bias associated with systemic error. The parameters controlled by the analyzer are:

A) specimen integrity and handling;

B) reagent and expendable availability and quality;

C) suitability and sensitivity of mechanical metering devices;

D) suitability and sensitivity of reaction inspection and measuring devices;

E) suitability and sensitivity of data analysis methods, wherein statistical quality control rule analysis of the control data allows for the monitoring of the system in statistical control, assuring the validity of the results; and

F) minimized bias when compared to reference methods.

In order to assure accurate laboratory assay results, it has always been necessary to devise quality assurance methods to monitor important variables of each of the above critical parameters. This type of monitoring is equally necessary for manual, semi-automated and automated analytical methods.

Prior to the present invention, skilled laboratory workers were responsible for monitoring these important variables for manual and semi-automated analytical methods using rudimentary off-line means. Some of these means included a traditional Levy Jennings approach to control ruling, visual inspection of samples for anomalies and no real-time monitoring of some instrument parameters was available. But the present invention requires on-line monitoring using sophisticated computer programming and integrated means.

Although each type of coagulation laboratory assay has specific critical parameters within the general parameters described above, some additional hemostasis and thrombosis assay critical parameters include:

1) Positive patient identification which includes bar code or similar identifier tracking, Delta check with previous specimen from the same patient; physiologic panic value evaluation; operational comments regarding the sample that follow data to the final report; and a statistical evaluation of replicate tests.

2) Preanalytical Variables are variables that can contribute to an anomalous result. Examples are specimen age, plasma with clot contamination, the amount of anticoagulant present in the blood collection tube to plasma collected ratio, nonanalyte interferences, optimized thermal storage of specimens to offset degradation with activation, hemolysis of sample, bilirubin content, and lipemic samples.

3) Sampling from a primary specimen container that allows for repeated testing from the same closed container without plasma carryover effects or aerosolization of the blood samples.

4) Reagent and Expendable Availability—a broad diagnostic assay menu supported with the proper reagents, which are monitored, tracked and flagged for the operator when the levels are low; a liquid level sensor on the probe via, for example, capacitance touch facilities; flagging failure of the analyzer to dispense; logic programming omitting the performance of an assay if sufficient reagents not available; bar code or similar identifier identification of the actual placement of a reagent in reagent tray; and preloaded cuvette cassettes handling a large number of cuvettes insure the optical clarity of the cuvette by minimizing handling of cuvettes.

5) Reagent quality is insured by refrigerated storage; by a reagent tray cover that minimizes evaporation and condensation; by the tracking of expiration dates; by tracking of reagent quality within each run, day-to-day, and month-to-month via an on-board quality control program using controls; by an assay specific quality control program which

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employs statistical rules with the ability to detect errors in the reagents; by tracking reagent quality independently of biological control plasmas using averaged patient data parameters; by normalizing minor drifts in reagent viability over time by assay calibration using calibrator plasmas; and by stirring reagents requiring stirring to maintain homogeneous suspension.

As can be seen from the above descriptions of the various segments of the automated coagulation analyzer, the segments are interdependent. Each of the four segments, specimen handling, sample preparation, optical inspection and signal processing are monitored and regulated and checked by on-board quality assurance and quality control program, providing an oversight function for all critical parameters. Each of these segments will be now more fully discussed along with the integration of these segments with the quality control and quality assurance features of the automated coagulation analyzer.

Referring to FIG. 1, there is shown an optical evaluation instrument incorporating a sample handling system according to the invention. The principal elements of the sample handling system include a cuvette storage and loading mechanism 10, for supplying cuvettes individually to a cuvette transport mechanism 12, which advances the cuvettes along a linear track 13 through a sample insertion station 14, a plurality of reagent insertion stations 16, 18 and 20, an optical monitoring station 22 and finally, to a cuvette disposal station 24. The sample handling system additionally includes a refrigerated housing 26, for storing a plurality of evacuated collection tubes (not shown), which are transported via shuttles 28 through a programming station 30, including a bar code reader 32, for reading a preprinted bar code printed on the side of each evacuated collection tube identifying the test sample and the test to be performed, and onto sample insertion station 14, which includes a piercer 34, for piercing the septum of an evacuated collection tube for allowing a sample probe 36 (see FIG. 2) to be lowered into the sample collection tube for aspirating a fluid sample which is to be ejected into a reaction well of a cuvette located at sample insertion station 14, as described in greater detail herein below. Refrigerated housing 26, additionally encloses a reagent chamber 35, which stores a plurality of reagent containers (see FIGS. 5 and 6), which can be accessed by reagent probes 38, 40 and 42 (see FIG. 2), for aspirating selective reagents and injecting them into reaction wells located at the respective reagent insertion stations 16, 18 and 20. As used herein, reagents include any reagent, diluent, buffer, or activator which is required for any given biochemical test being performed on the patient sample according to a preprogrammed test protocol. A probe washing station 44, is provided for washing the sample and reagent probes after each dispensing action.

Referring to FIGS. 1 and 4, cuvette storage device 10 includes a cassette frame 46, for receiving a cassette of cuvettes arranged in the cassette in columns parallel to the right and left hand sides of frame 46 in FIG. 1. The cassettes are preferably of the type described in U.S. Pat. No. 5,040,894 to Karp et al. cited above. A plan view of one such cuvette 50 is seen in a loading position with respect to cuvette transport mechanism 12. A pusher arm 52, driven by a lead screw 54, loads cuvettes onto cuvette transport mechanism 12. A motor 56, whose shaft 58, is connected with a pulley 60 rotates a driving belt 62 which turns a pulley 64 for driving lead screw 54. A fixed guide rod 55 is provided in the usual manner for providing guidance and additional support for pusher arm 52. After a column of cuvettes is completely loaded onto cuvette transport mecha-

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nism 12, pusher arm 52 is retracted and a new column of cuvettes is moved rightwardly (in FIG. 1) by way of a cassette column drive mechanism to be in line with pusher arm 52. The cassette column drive mechanism includes a plate 66, provided with fingers 68, extending through slots 70 in a bottom support 72 of cassette frame 46. A rectangular plate (now shown), is positioned between fingers 68 and the left-hand most column of cassettes (not shown) in frame 46 for pushing the cassette columns in a rightward direction in FIG. 1. Plate 66 is driven by way of a motor 74 whose shaft 76 is connected to a pulley 78 which turns a driving belt 80 connected to a further pulley 82 which turns a lead screw 84, whose threads engage with plate 66. A fixed guide rod 86, is provided parallel to lead screw 84, for guiding plate 66 in the usual manner.

Cuvette transport mechanism 12, includes a lead screw 88, which is driven by way of a motor 90 whose shaft 92 is connected to a pulley 94 for turning a belt 96 which is connected for driving a pulley 98 connected to lead screw 88. The cuvettes are each provided with an engaging means, such as a rib having the same pitched angle as the threads of lead screw 88 which engage the lead screw threads when placed in a loading position by pusher arm 52. A cuvette 50 is shown in the loading position engaging lead screw 88. Cuvettes of this type, which desirably have four reaction wells, as shown by cuvette 50, are disclosed in the aforementioned U.S. Pat. No. 5,040,894, to Karp et al. Once engaged with lead screw 88, the cuvettes are advanced in a rightward direction in FIG. 1 along linear track 13 through the various stations as described herein for injecting a sample volume and reagents to create a reaction volume to be optically monitored at the optical monitoring station.

Linear track 13 is preferably made of a single piece of aluminum having a smooth upper surface on which the cuvettes can slide without interference. Desirably, linear track 13 is temperature controlled for controlling the temperature of the contents of the cuvette reaction wells, which contents are in heat exchange relationship with the track by way of the cuvettes. For this purpose, linear track 13 is cooled on the left side of a heat flow restriction 15 shown in FIG. 2 by way of, for example a Peltier device (not shown) to maintain the temperature of the reaction well contents at about 15° C. On the right hand side of heat flow restriction 15, linear track 13 is heated by way of a heating element 17, such as a resistive heat tape, applied to the under side of the linear track for maintaining the temperature of the reaction well contents at body temperature. Preferably, heat flow restriction 15 is formed by an elongated notch in the underside of linear track 13 so as to reduce the cross section of the track in the region of the notch and thus correspondingly reduce the heat flow by an effective amount from the heated portion to the cooled portion of the track. The upper surface of the track in the region of the notch remains smooth and continuous so as not to present any interference with the cuvettes sliding thereon. Control signals for controlling motors 56, 74, and 90, for turning respective lead screws 54, 84 and 88, to accomplish the required incremental movements of pusher arm 52, plate 66, and cuvette 50, respectively, are received from a central controller (not shown) of the instrument in a manner well understood by those skilled in the art.

Sample probe 36, and reagent probes 38, 40 and 42 are controllably moved along a horizontal path by way of respective lead screws 100, 102, 104 and 106, driven by respective motor assemblies 108, 110, 112 and 114. Vertical movement for lowering and raising sample probe 36, and reagent probes 38, 40 and 42, is accomplished by way of

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respective vertical gear racks **116**, **118**, **120** and **122**, driven by corresponding vertical motor and pinion assemblies **124**, **126**, **128**, and **130**, respectively. Horizontal lead screw motors **108**, **110**, **112** and **114**, and vertical rack and pinion motors **124**, **126**, **128** and **130**, are selectively controlled by signals received from the instrument controller (not shown) for controlling the horizontal and vertical movement of the respective probes for aspirating and dispensing sample and reagents according to the test protocol identified from the bar code of a given sample collection tube read by bar code reader **32**. Sample and reagent aspiration and dispensing by probes **26**, **28**, **30** and **32** is accomplished by way of positive displacement pumps (not shown) connected to the respective probes in a manner understood by those skilled in the art.

Refrigerated housing **26**, comprises a double walled insulated enclosure **131**, and a cooling system **133**, preferably of the ducted type, for circulating cooling air within housing **26** for maintaining the temperature of sample collection tubes (not shown) mounted in shuttles **28** and reagents in reagent chamber **36** at a temperature between 4° and 8° C.

Referring to the plan view shown in FIG. 1, refrigerated housing **26**, has left and right chambers **132** and **134**, respectively, connected by passages **146** and **147** for storing and transporting shuttles **28**, which are caused to move in a clockwise direction, as shown by arrows **135** to **138**. Each shuttle **28** is provided with means for carrying a plurality of evacuated sample collection tubes of the type, for example, made by Beckton Dickinson of Rutherford, N.J., and sold under the brand name Vacutainer. The configuration of shuttles **28**, and the mechanism for transporting the shuttles is disclosed in detail, for example, in U.S. Patent No. **3,418,084** to Ailington.

Briefly, each shuttle **28** has complimentary camming surfaces **140** and **142** formed at the opposite ends thereof. Shuttles **28** are disposed in rows in the respective chambers **132** and **134**. A drive mechanism (not shown) comprising gears which mesh with gear tracks **29** on the bottom of the shuttles **28** (FIG. 2), drive the shuttles through passages **146** and **147** in opposite directions. The shuttle drive mechanism causes a driven shuttle to push the shuttle in front of it and the camming surfaces effect a lateral displacement in the manner described by the above-referenced patent to Ailington. The shuttles are transported, one behind the other, in passages **146**, so that the evacuated collection tubes are passed first through programming station **30** where bar code reader **32** reads a previously-applied bar code on the side of the evacuated collection tube to identify the sample and the test to be performed. The information read by bar code reader **32** is fed to the instrument controller (not shown) for controlling subsequent movement of the sample and reagent probes for filling a reaction well of a cuvette transported by cuvette transporting mechanism **12** through the respective sample and reagent stations.

After having its bar code read, the evacuated collection tube is moved, by way of the shuttle and shuttle drive mechanism, a precise distance to place the evacuated collection tube in line with piercer **34**. The precise positioning of the shuttle is accomplished by way of an electro-optical sensing mechanism **148** (FIG. 1), which passes a sensing beam through spaced passages **150** (FIG. 2), provided in the base of shuttles **28**, for sensing when the shuttle is in the appropriate position.

Referring to FIG. 3, piercer **34** includes a piercing tube **152** having a sharp angled end **154**, canted at approximately the same angle as the tip of a conventional hypodermic needle, for piercing a septum **156** of an evacuated collection tube **158**. Piercing tube **152** is mounted in a support **160**

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which engages a vertical lead screw **162** which is connected by way of a belt and pulley system **164** to a motor **166** for driving lead screw **162**. With appropriate movement of lead screw **162**, piercing tube **152** is caused to be lowered for piercing septum **156** or to be removed therefrom. A holding mechanism **159** holds tube **158** in place while piercing tube **152** is inserted and withdrawn. Piercer **34** has an opening **168** (FIG. 1) at the top concentrically aligned with piercing tube **152**, so that when sample probe **36** is aligned with piercing tube **152** a pathway is provided for lowering the sample probe into an evacuated collection tube **158**, for aspirating a fluid sample therefrom.

Reagent chamber **35** is shown in greater detail in FIGS. 5 and 6. As shown in these Figures, a reagent container support plate or tray **170**, is provided for supporting a plurality of reagent containers or cups **172**, of varying capacities. A reagent cover **176**, of approximately one half inch thickness, is placed over reagent container support plate **170**. Reagent cover **176** is provided with reagent probe holes **178** positioned above respective ones of reagent containers **172**. Probe holes have a diameter (approximately 3 mm) slightly larger than the diameter of reagent probes **40** and **42** for permitting the probes to be lowered into selected ones of the reagent containers. Reagent cover **176** serves as an anti-evaporation cover for retarding or preventing evaporation of the reagents in reagent containers **172** while still allowing access to the reagents through probe holes **178**. The anti-evaporation cover additionally serves to retard rapid temperature shifts by providing a barrier between different temperature zones. Although there are multiple holes in the anti-evaporation cover, it is of sufficient depth to provide the tortuosity necessary to retard or prevent evaporation of liquids. Desirably, reagent cover **176** is provided with locator pins **180** for accurately positioning the cover over the reagent containers and in alignment with the horizontal tracks of reagent probes **38**, **40** and **42**.

Probe washing station **44**, comprises a trough **210**, containing a cleaning solution such as bleach. An additional trough **212** is provide for receiving waste fluids and cleaning solution from the probes during the washing process. Trough **212** is provided with a plurality of riser platforms **214**, **216**, **218** and **220**, each containing a concave recess and serving as a deflector for fluid and cleaning solution expelled from a probe. After a probe dispenses its fluid into a reaction well in a cuvette, and before the probe is positioned to aspirate sample or reagent as the case may be, the probe is positioned over trough **210** for aspirating cleaning solution. The probe is then positioned over the corresponding deflector where primer fluid, such as water, is forced through the probe interior for expelling the cleaning solution, followed by primer liquid, against the deflector thereby creating a fountain effect which washes the outside of the probe. The waste solutions are captured by trough **212** and vented away through a waste outlet (not shown). A more detailed description of probe washing station **44** is described in the aforementioned U.S. Pat. No. 4,989,623 to Hoffman et al.

Optical monitoring station **22** comprises a multichannel optical monitoring system of the type described in detail in the above-mentioned U.S. Pat. No. 5,002,392 to Swope et al. Briefly, and with reference to FIGS. 2 and 3, the optical monitoring system includes a broad band light source **182**, which passes light through a slit (not shown). A collimating lens **184**, collimates the beam to form a slowly diverging beam **186** which is folded by reflecting mirrors **188**, **190** and **192**. Following mirror **192** is a mask (not shown), which includes a plurality of linearly spaced apertures for dividing beam **186** into a corresponding number of beams, each

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defining an optical path or channel 194, as schematically illustrated in FIG. 2. The optical paths or channels 194 are linearly spaced along the track of cuvette transport mechanism 12 so that each reaction well of a cuvette passes from channel-to-channel as cuvette transport mechanism 12 incrementally advances the cuvettes along the linear path. The light beams passing through the reaction wells of the cuvettes are passed through a rotating shutter 194 which sequentially passes the light beams to diffraction gratings 196 where the beams are diffracted and focused by focusing lenses 198 onto respective photodiode arrays 200 which are subsequently electronically scanned for reading electronic signals which correspond to the spectral distribution of the beams transmitted by the respective reaction volumes contained in the reaction wells of the cuvettes. In one specific implementation, the optical monitoring system includes 20 channels, and the rotating shutter operates to sequentially pass the beams within groups of five beams, so that only one beam from each group of five beams is passed onto a photodiode array at any one time. The focusing lenses operate to focus each group of five beams onto a respective one of photodiode arrays 200.

The invention will now be described more particularly in connection with coagulation, chromogenic and immunological testing.

I. Specimen Handling

The present invention automatically handles the sample to be tested from the moment the collection tube containing the sample is placed in the analyzer. The analyzer has a first transporting device for transporting the sample collection tubes, in order, first to the programming station and then to the sample insertion station and a second transporting device for transporting the cuvettes through the sample insertion station, the reagent station and on to the optical monitoring device where the optical characteristics of the reaction volume in the respective reaction wells can be monitored.

According to a preferred embodiment of the invention, the sample collection tubes are evacuated and sealed by a septum, the tubes are sampled with a piercing/aspirating sample probe which deposits the proper amount of sample into the well of a cuvette. The preferred cuvette is described in Karp et al. US Des. 325,090 and U.S. Pat. No. 5,040,894.

According to another aspect of the invention, the temperature controlled housing maintains the temperature of the evacuated collection tubes and the reagent containers between 9° C. and 15° C.

Further, the second transporting device preferably includes a linear track for guiding the cuvettes and a drive mechanism for periodically moving the cuvettes along the track in discrete increments. Preferably, the drive mechanism includes a lead screw and the cuvettes are each shaped for engaging the lead screw for being driven along the linear track in the manner described in the above referenced U.S. Pat. No. 5,040,894. According to yet a further aspect of the invention, the cuvette storage includes a device for removing the cuvettes from the storage and placing the cuvettes onto the linear track.

Additionally, the first transporting device preferably includes a plurality of shuttles each for holding a plurality of sample collection tubes and means for moving the shuttles through the programming and sample insertion stations.

Each sample is given a machine-readable identification, such as a bar code, which is used in assay entry and during tracking of the assay. For example, a freshly drawn tube of blood is manually labelled with a bar code, and the bar code, patient identification and required tests are manually entered into the analyzer's computer. The tube is then placed into a

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shuttle and the shuttle placed in the shuttle storage area, where its bar code is automatically read by the analyzer and tracked by the quality control programming. The septum is pierced with the sampling probe, called Probe 1, which has been programmed as defined in the adf, to withdraw a specified amount of sample from the tube or collection device, which proceeds to aspirate the sample and dispense it into a predetermined well of a predetermined cuvette. The probe is then washed in order to remove essentially all of the sample remaining on it, in order to avoid cross-contaminating the next sample. Wash solutions include, among others, water, bleach solutions, preferably a 10% bleach solution, or specifically formulated wash solutions that are capable of removing essentially all of the sample from the probe. At times later in the assay, each probe can be washed after each use, for the same reasons. However, another reason for washing a probe is because in these types of assays, should thrombin, thromboplastin or phospholipids contaminate the probe, they are extremely difficult to remove, and thereby contaminate the next assay well. Removal of these extremely sticky substances from the probe is needed for both the method and the instrument to perform.

The analyzer has been programmed so that this particular well will have a specific assay performed in it. The quality assurance program tracks the well at specified times throughout the test procedure, confirming assay identification, correct volume delivery, correct adf interpretation and proper temperature control at critical times.

Preanalytical variables, such as the presence of hemolysis, bilirubin, lipemia, and fibrin clots are determined in conjunction with the performance of an assay. This is accomplished by utilizing a bichromatic technique inspecting a baseline wavelength and wavelength where the substance of interest can be detected. Unique algorithms are then applied to quantitate each substance. For example, hemolysis is detected by monitoring transmittance at 535 nm and 515 nm and computing a hemolysis index, which is

$$\text{Hemolysis Index (HI)} = \frac{\text{Normalized transmittance at 535 nm}}{\text{Normalized transmittance at 515 nm}}$$

The heme unit of the hemoglobin molecule has adsorption at the 535 nm band pass (see FIG. 1).

Bilirubin is conducted the same way but uses 450 nm and 710 nm as the wavelengths of interest, while lipemia is measured by monitoring the normalized transmittance at 710 nm.

II. Sample Preparation

The next step is the automatic preparation of the sample for testing. This includes the ability of the analyzer to access any reagent and to deposit it in a cuvette well; to wash the probe through which the reagent is accessed after each reagent is dispensed with a wash solution as described above in order to avoid cross-contamination problems between reagents or reagents and samples; a universal profile testing method for all coagulation assays; a means for automatically diluting the sample; and a means for monitoring levels of the reagents and samples in their cuvettes and tubes.

The random access movement of the probes is used to aspirate and dispense reagents and samples according to test protocol for a particular assay (defined by the adf parameters), ordered or scheduled from the bar code of a given sample collection tube. This movement, and a description of the analyzers instrumentation, is more fully explained in the co-pending parent application, U.S. Ser. No. 07/833,950. Correct reagent/plasma volume delivery is monitored through the use of a novel dye tracking system,

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one type of which is described in U.S. Pat. No. 5,068,181. The versatility provided by the presence of adf's allow the fully automated analyzer to be optimized for each specific assay, allowing for the flexibility required for radically different assay formats. Below are diagrams of adfs for three assays (Tables 1–3), Factor VIII reference curves, PT and plasminogen reference curves. The first column notations A1, A2, A3 and A4 refer to the automated arms of the

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analyzer, the second column notations refer to various operations, such as pumping velocity, dilution, and aspiration. The numbers after the colons are the editable parameters, dealing with each of the various operations, such as volume amount or number of dilutions or velocity. A number of 000 means that no function is performed by the particular arm at that particular point in time. The notations after A4 refer to the optical set-up of the analyzer.

TABLE 1

adf Parameters for FVIII Ref. Curves			
A1 Run Volume	: 50	A3 Reagent Volume	: 051
A1 Pump Velocity	: 1000	A3 Reagent Airslug	: 040
A1 Sample Location	: 001	A3 Pump Velocity	: 1250
A1 Sampling Macro	: SFSTASP	A3 Reagent Location	: 001
A1 Dilution Count	: 007	A3 Fetch Macro	: FETCH2
A1 Dilution Mix (1:x)	: 010	A3 Deliver Macro	: DELREAGENT
A1 Dilution Sample	: 010	A3 Wash/Rinse Macro	: WASH
A1 Dilution Primer	: 090	A3 Cleaner Volume	: 005
A1 Dilution Aspirate	: 050	A3 Cleaner AirSlug	: 085
A1 Dilution Macro	: SMIDASP	A3 Rinse Volume	: 800
A1 Non-Serial Dilution Count	: 000		
A1 Wash/Rinse Macro	: RINSEP	A4 Reagent Volume	: 051
A1 Cleaner Volume	: 000	A4 Reagent AirSlug	: 040
A1 Cleaner AirSlug	: 000	A4 Pump Velocity	: 1500
A1 Rinse Volume	: 800	A4 Reagent Location	: 002
		A4 Fetch Macro	: FETCH2
A2 Dilution Volume	: 051	A4 Deliver Macro	: DELOPTICS
A2 Primer Volume	: 000	A4 Wash/Rinse Macro	: WASH
A2 Aspirate Volume	: 000	A4 Cleaner Volume	: 005
A2 Dilution AirSlug	: 010	A4 Cleaner AirSlug	: 085
A2 Pump Velocity	: 1000	A4 Rinse Volume	: 800
A2 Buffer Location	: 005	Assay Blank time	: 007
A2 Dilution Macro	: FETCHOEL	Assay Maximum Time	: 240
A2 Dilution Count	: 000	Normalization Value	: 3000
A2 Dilution Concentrat	: 000	Replicate Count	: 000
A2 Dilution Mix (1:x)	: 000	Wavelength Count	: 003
A2 Non-Serial Dilution Count	: 000	5, 15, 20	
		Dltn Recalc Override	: 0000
A2 Throw Away Volume	: 000	Spare 2	: 0000
A2 Middle Dilution	: 000	Spare 3	: 0000
A2 Middle Primer Vol	: 000	Spare 4	: 0000
A2 Middle Aspirate Vol	: 000	Spare 5	: 0000
A2 Middle Dilution Macro	:		
A2 Wash/Rinse Macro	: RINSEP	ADF Name	: FVIIIIR.ADF
A2 Cleaner Volume	: 000		
A2 Cleaner AirSlug	: 000		
A2 Rinse Volume	: 800		

TABLE 2

adf Parameters for PT			
A1 Run Volume	: 51	A3 Reagent Volume	: 000
A1 Pump Velocity	: 1000	A3 Reagent Airslug	: 000
A1 Sample Location	: 001	A3 Pump Velocity	: 0000
A1 Sampling Macro	: PIEREC	A3 Reagent Location	: 000
A1 Dilution Count	: 000	A3 Fetch Macro	:
A1 Dilution Mix (1:x)	: 000	A3 Deliver Macro	:
A1 Dilution Sample	: 000	A3 Wash/Rinse Macro	:
A1 Dilution Primer	: 000	A3 Cleaner Volume	: 000
A1 Dilution Aspirate	: 000	A3 Cleaner AirSlug	: 000
A1 Dilution Macro	:	A3 Rinse Volume	: 000
A1 Non-Serial Dilution Count	: 000		
A1 Wash/Rinse Macro	: RINSEP	A4 Reagent Volume	: 101
A1 Cleaner Volume	: 000	A4 Reagent AirSlug	: 040
A1 Cleaner AirSlug	: 000	A4 Pump Velocity	: 1250
A1 Rinse Volume	: 800	A4 Reagent Location	: 001
		A4 Fetch Macro	: FETCH3
A2 Dilution Volume	: 000	A4 Deliver Macro	: DELOPTICS3
A2 Primer Volume	: 000	A4 Wash/Rinse Macro	: WASH1
A2 Aspirate Volume	: 000	A4 Cleaner Volume	: 030
A2 Dilution AirSlug	: 000	A4 Cleaner AirSlug	: 115
A2 Pump Velocity	: 0000	A4 Rinse Volume	: 800
A2 Buffer Location	: 000	Assay Blank time	: 005

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TABLE 2-continued

adf Parameters for PT			
A2 Dilution Macro	:	Assay Maximum Time	: 150
A2 Dilution Count	: 000	Normalization Value	: 3000
A2 Dilution Concentrat	: 000	Replicate Count	: 000
A2 Dilution Mix (1:x)	: 000	Wavelength Count	: 003
A2 Non-Serial Dilution Count	: 000	5, 15, 20	
		Dltm Recalc Override	: 0000
A2 Throw Away Volume	: 000	Spare 2	: 0000
A2 Middle Dilution	: 000	Spare 3	: 0000
A2 Middle Primer Vol	: 000	Spare 4	: 0000
A2 Middle Aspirate Vol	: 000	Spare 5	: 0000
A2 Middle Dilution Macro	:		
A2 Wash/Rinse Macro	:		
A2 Cleaner Volume	: 000	ADF Name	: PT.ADF
A2 Cleaner AirSlug	: 000		
A2 Rinse Volume	: 000		

TABLE 3

adf Parameters for Plasminogen			
A1 Run Volume	: 50	A3 Reagent Volume	: 050
A1 Pump Velocity	: 1500	A3 Reagent Airslug	: 005
A1 Sample Location	: 001	A3 Pump Velocity	: 1250
A1 Sampling Macro	: SFSTASP2	A3 Reagent Location	: 004
A1 Dilution Count	: 000	A3 Fetch Macro	: FETCH1
A1 Dilution Mix (1:x)	: 000	A3 Deliver Macro	: DELREAGENT
A1 Dilution Sample	: 010	A3 Wash/Rinse Macro	: WASH
A1 Dilution Primer	: 010	A3 Cleaner Volume	: 010
A1 Dilution Aspirate	: 000	A3 Cleaner AirSlug	: 045
A1 Dilution Macro	:	A3 Rinse Volume	: 800
A1 Non-Serial Dilution Count	: 000		
A1 Wash/Rinse Macro	: RINSEP	A4 Reagent Volume	: 050
A1 Cleaner Volume	: 000	A4 Reagent AirSlug	: 040
A1 Cleaner AirSlug	: 000	A4 Pump Velocity	: 1500
A1 Rinse Volume	: 800	A4 Reagent Location	: 004
		A4 Fetch Macro	: FETCH1
A2 Dilution Volume	: 040	A4 Deliver Macro	: DELOPTICS
A2 Primer Volume	: 140	A4 Wash/Rinse Macro	: WASH
A2 Aspirate Volume	: 155	A4 Cleaner Volume	: 010
A2 Dilution AirSlug	: 000	A4 Cleaner AirSlug	: 080
A2 Pump Velocity	: 1600	A4 Rinse Volume	: 800
A2 Buffer Location	: 003	Assay Blank time	: 005
A2 Dilution Macro	: BFSTRA	Assay Maximum Time	: 060
A2 Dilution Count	: 002	Normalization Value	: 3000
A2 Dilution Concentrat	: 005	Replicate Count	: 000
A2 Dilution Mix (1:x)	: 020	Wavelength Count	: 003
A2 Non-Serial Dilution Count	: 001	2, 3, 34	
		Dltm Recalc Override	: 0001
A2 Throw Away Volume	: 100	Spare 2	: 0000
A2 Middle Dilution	: 010	Spare 3	: 0000
A2 Middle Primer Vol	: 040	Spare 4	: 0000
A2 Middle Aspirate Vol	: 050	Spare 5	: 0000
A2 Middle Dilution Macro	: BMIDASP		
A2 Wash/Rinse Macro	: RINSEC		
A2 Cleaner Volume	: 000		
A2 Cleaner AirSlug	: 000		
A2 Rinse Volume	: 800		

The universal testing profile is an extremely important part of this invention. All known coagulation analyzers, semi-automated and automated, perform on the basis that each test requires a unique profile. For example, only Prothrombin (PT) or only APTT assays are to be run at any given time as a batch, and therefore such machines are programmed to run the same way for each sample. They are incapable of doing a prothrombin time test on one sample and an APTT on the next, as the temperature vs. time parameters of each test are different. Some analyzers can perform multiple batch analysis simultaneously, but do not use the same profile. One way of doing so is being having unique pathways for each batch mode. The universal throm-

bosis and hemostasis temperature profile (temperature vs. time) is a method that allows all coagulation assays to be run through the same linear transport system using the same timing sequence, on the analyzer, to produce the necessary profile, with variables being the probe delivery temperatures and volumes, which are functions of parameters established by the adf.

The universal profile represents a balance between the different thermal requirements for each assay. This allows coagulation assays to be performed in a continuous, fully automated format.

There are nine critical requirements of the preferred universal thrombosis and hemostasis profile:

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1. The temperature of the sample dispensed into the cuvette is preferably between about 4° C. and about 25° C.

2. The sample must remain in a temperature range of from about 4° C. to about 25° C. until the heating sequence begins.

3. The time required to warm the sample temperature from the pre-ramp temperature range to the post-ramp temperature range is 80±10 seconds.

4. The temperature of the sample at the end of the ramp must be 33° C.±1° C.

5. If the test is an APTT, the activation reagent is added immediately at the end of the ramp (Arm 3).

6. The temperature of the reagent delivered at R1 is 40° C.±1° C., raising the reaction temperature to 37° C.

7. If the test is a PT, the sample must reach 37° C.±1° C. in 120±10 seconds after the 80 second ramp segment (Arm 4).

The temperature of the reagent delivered at R2 is 37° C.±1° C.

9. The temperature of the mixture must remain at 37° C.±1° C. for at least 360 seconds after R2.

These temperature and time controls occur due to the interaction of the heated probes that aspirate and dispense liquids, which are described in more detail in U.S. Pat. No. 5,178,019 to Keiter, and the portion of the sample handling system, the heating and cooling track, as described in U.S. Ser. No. 07/833,950.

Flexibility in the adf allows for minor changes in temperature profiles that allow for the total optimization of each assay. Diagrams of the profile and optimization ranges for the PT and APTT assays are attached (see FIG. 8).

Another aspect of the sample preparation segment of the automatic coagulation analyzer is a means to automatically dilute samples. The analyzer performs a wide range of serial and nonserial dilutions for a large set of assays in a real time format. The adf for each complex method (methods requiring dilutions) defines the dilution: serial and non-serial, which buffers to use, how to dilute concentrated buffers and which arm to perform the dilution on (arm 1 or 2). Successful dilutions are a function of fluidic movement, probe design and robotic design. In addition, the analyzer, with its built-in quality control and quality assurance programming, ensures the accuracy of the dilutions. FIG. 9 shows the relative absorbance rates of a dye sample, serially diluted. A least squares regression line is also shown, and its goodness-of-fit measure r^2 .

Finally, the analyzer provides a means for monitoring levels of the reagents in their containers and of the samples in their sample tubes or holding devices. This is another necessary function for a fully automated analyzer, as an assay cannot be performed without the proper amount of reagents. In the present analyzer, a reagent chamber holds a large number of reagent containers, at a temperature of about 7° C., and some reagents may be automatically mixed to maintain a homogeneous suspension. If necessary, the reagent is heated in the probe before dispensing. Fluid level sensing is used to control the height of a reagent probe relative to the level of a reagent in its container.

III. Optical Inspection

Once the reagents are added to the test sample, the reaction, if any, is read through spectrophotometric means. The optical inspection segment of the analyzer consists of a means for multiple wavelength analysis; a means to continuously normalize the fluctuations in light levels associated with sample to sample variability; and a means for using a broad spectrum of wavelengths in order to read the results of a variety of test reactions at the appropriate wavelength for that test.

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As stated above, a preferred format of these functions are well-described in U.S. Pat. No. 5,002,392 and U.S. Ser. No. 07/869,579.

The quality control programming insures proper wavelength selection by monitoring the signal noise to ratio for each sample as defined in the adf. If the signal to noise threshold is exceeded then another wavelength is used. If all wavelengths are unacceptable then an error flag is provided to the user.

The quality assurance programming assures that the true wavelength is being evaluated by incorporating a piece of spectral glass with known absorbance characteristics. The peaks are measured by the analyzer and compared to the known values. Furthermore, a liquid crystal clot simulator has been incorporated into the optics module that when properly stimulated creates a signal that is similar to a clot. The output is inspected to insure the integrity of the optics unit as well as the integrity of the analysis algorithms.

IV. Signal Processing

The signal processing segment of the analyzer is the segment that reports the results of the assays. The analyzer has the ability to determine kinetic endpoints; to do multi-rule analysis of the final test results; to perform complex processing that determines endpoints other than clot formation; and provides an on-line database against which each test result can be compared.

An endpoint algorithm library is required to facilitate the analysis of the different types of assays. The major categories of endpoint analysis are linear rates, logarithmic rates, relative magnitudes and kinetic endpoints based on the biological characteristics of multianalyte mixtures (i.e., clot formation).

For example, the standard way of determining a PT or APTT clotting time is to take a sample, add the appropriate reagents and visually or spectrophotometrically determine when a clot has formed. When visually inspecting a sample for clot formation, the sample will turn more turbid after a clot has formed. Semi-automated analyzers set an endpoint at an arbitrary level of light reduction that is somewhat equitable to the visual method. However, this method is susceptible to error due to the presence of mechanical, optical, electrical and biological artifacts. Furthermore, as the instrument "ages", decreases in light transmission can result in a shift in the endpoint times.

Visual clot formulation occurs when the specimen mixture turns turbid. This can be physiologically associated with the initial conversion of fibrinogen to fibrin. (See, E. Ludvig, "Perception of Contour," Bureau of Medicine and Surgery, Proj. No. NM001 075.01.04, Aug. 17, 1953.) The maximum rate of acceleration, the time of the peak of the second derivative, is indicative of the initial fibrinogen, fibrin conversion and directly correlates to a visual method (see FIG. 11). Because the time is computed on a data stream that is independent of arbitrary thresholds the usual artifacts associated with the instrument and biologics are negated resulting in more accurate and precise results. The same approach of analyzing characteristics of the signal stream for unique parameters is applied to the calculation of rate and magnitude for chromogenic, immunologic and quantitative fibrinogen assays.

For complex assays, kinetic endpoint results, such as clot times and reaction rates, additional processing is required. In these assays, the desired reported value of a sample is a function of some reference curve, which relates a quantity of a known analyte to reported endpoint values. Typically, a series of endpoint-based tests are performed on a series of concentrations from dilutions of a reference material. The

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paired data sets of recovered and reported values are then used to construct the reference curve.

The reference curve represents a function which is a model built using the sample data. (See, "Computer Evaluation of Reference Curves for the Estimation of Extrinsic Coagulation Factors," Frank et al., *Comput Biol Med*, Jan 1978). Traditionally, these functions have been assumed to be of a form similar to

$$f(\text{recovered value}) = a_1 g(\text{reported value}) + a_0$$

where $f()$, $g()$ are typically $\log_{10}()$. This function is particularly easy to represent graphically by hand, and numerically the coefficients can be recovered by simple linear regression using the formulas,

$$\text{slope} = \frac{n \sum xy - (\sum x)(\sum y)}{n \sum x^2 - (\sum x)^2}$$

$$\text{intercept} = \bar{Y} - \text{slope} \bar{X}$$

This type of function, while seemingly accurate, especially when calculated graphically by hand, is not representative of the true biological processes and leads to errors because points deviating from a straight line on a log-log reference curve are thought to deviate by random error and are neglected.

These simple functions restrict the degrees of freedom necessary to accurate model coagulation assays, which are more complex than simple chemical determinations based on known formulas. There are agents present in addition to the analyte of interest that complicate the cause-effect relationship.

The methods available in this invention allow for more flexibility in designing a model of the reference function which relates the desired reported results with recovered values. These functions provide a more accurate representation of the shape of the reference curve for each assay, accommodating unknown extraneous agents, which leads to more accurate and precise results; also provide robustness in the presence of small errors in the data; gives accurate representation of all sample data, without neglecting points; and providing for an increased range of reference recovery due to incorporation of all dilutions, including those that were once ignored due to deviation from the straight line.

Three methods that are generally used to create models from experimental data are: (1) Gauss Jordan and (2) Singular Value Decomposition (SVD) for linear systems, and (3) Levenberg-Marquardt Method for non-linear systems. Gauss-Jordan is generally considered a simpler, but less accurate method for solving linear systems. Singular-Value is more costly in terms of number of operations, but it has the advantages of being more accurate, and is robust in the presence of singularities, where Gauss-Jordan cannot function. These singularities are approached when there are two solutions very close together, which is not uncommon. The error creeps into Gauss-Jordan when two very large numbers cancel each other when there is a very small pivot (i.e., close to singular). The accuracy of SVD and Levenberg-Marquardt methods are comparable.

The general form of a linear (in its coefficients) data model is

$$y(x) = \sum_{k=1}^M a_k X_k(x).$$

The merit function typically used to determine the best set of parameters a_k is

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$$\chi^2 = \sum_{i=1}^N \left[\frac{y_i - \sum_{k=1}^M a_k X_k(x_i)}{\sigma_i} \right]^2.$$

The minimum of this function is found where the derivative of χ^2 with respect to all M parameters $a_k=0$, which leads to what are known as the normal equations, or a linear system

$$\sum_{j=1}^M \left[\sum_{i=1}^N \frac{X_j(x_i) X_k(x_i)}{\sigma_i^2} \right] a_j = \left[\sum_{i=1}^N \frac{y_i X_k(x_i)}{\sigma_i^2} \right]_k.$$

In order to provide flexibility in determining the optimum reference function for each assay, several components were made available in the complex data analysis module:

A variety of data transformations, i.e., \log_{10} , $1/x$, etc.;

switching the x and y variables;

Polynomials of any order; and

Piecewise fitting, including overlapping.

The general form of the functions is

$$f(y) = c_0 + c_1 f(x) + c_2 f(x)^2 \dots c_n f(x)^n$$

where

$$f(x) = x, \frac{1}{x}, \frac{1}{f(x)}, \log(x) \dots$$

The "piecewise" form is

$$f(y) = [f_1(x)]_{x=a}^{x=b} [f_2(x)]_{x=b}^{x=c} \dots [f_n(x)]_{x=g}^{x=h}$$

where each "subfunction" can be a different type base on any combination of the sample data. Having the ability to select from the above options, an optimum reference function was selected for each complex assay. These optimized reference functions improve both the accuracy and precision of the assays. Each method was tested to determine if samples with known reported values were reported accurately, if diluted samples resulted in reported values at the expected levels, and if the sample data were fit well (R^2).

V. Quality Assurance

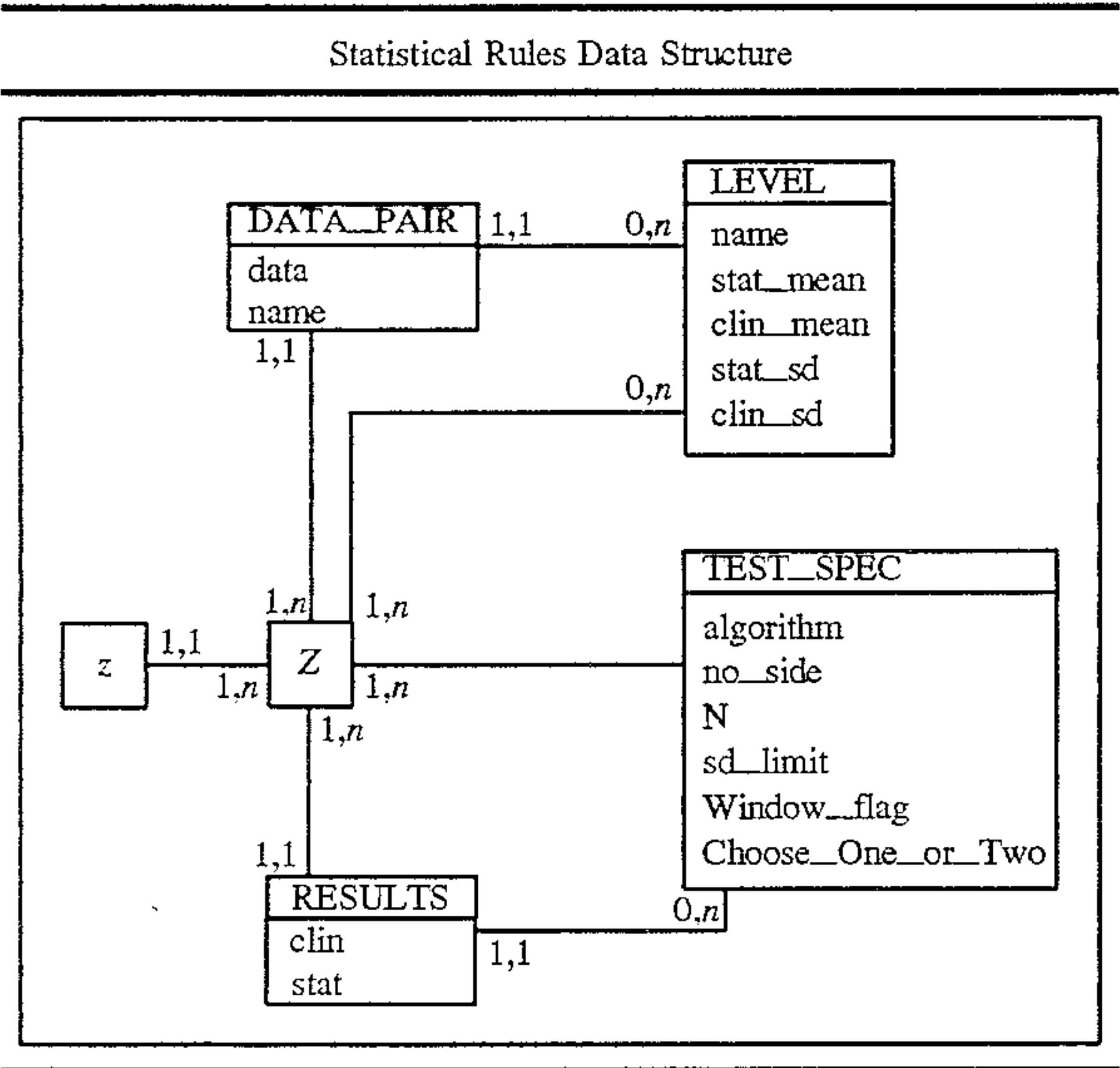
An important part of the quality control package is the use of statistical quality control rules applied to test data. This is very similar to the tests originally utilized for clinical chemistry quality control programs, known as the Westgard multirule systems but that have been applied to coagulation assays (See, Westgard, J., Wiebe, D., "Cholesterol Operational Process Specifications for Assuring the Quality Required by CLIA Proficiency Testing," *Clinical Chemistry*, Vol. 37, No. 11, pp. 1938-1944, 1991; and Westgard, J., Peterson, P., Wiebe, D., "Laboratory Process Specifications for Assuring Quality in the U.S. National Cholesterol Education Program," *Clinical Chemistry*, Vol. 37, No. 5, pp 656-661, 1991). This is available for use in two portions of the analyzer, the system quality control program and the on-line test quality control.

The system quality control defaults to a new control run mode when the allotted time designated for control run frequency is reached. The system pauses for the control run, which can only be overridden when an emergency sample is requested. Once the controls are assayed, the statistical quality control rule chosen is activated to interpret the results of the controls. If data passes the rules, quality control results are printed on a hard copy while being simultaneously saved to the on-board computer. If data

violates any of the rules, it is flagged through a hard copy and the analyzer's monitor. The user has an option to either repeat the control, troubleshoot the cause of the flag or accept flagged control values.

A preferred variation of statistical rule data structure is shown in the following Table 4.

TABLE 4



The purpose of the statistical quality control rules is to take an array of raw data (which may include data from several different controls or levels) and apply a set of tests to it. The set of tests, if chosen properly can have a very low false rejection rate coupled with a very high probability of rejection of bad results. Before the tests are applied to the data, they are converted to z-scores (normalized) using the level data, allowing different controls to be analyzed together. There can be any number of tests in a statistical rule, and a pair of results is returned for each individual test: one based on clinically significant ranges and one based on statistically significant ranges. Any number of raw data points may be used, as the rules assume that all of the data is to be examined. If GROUP window_flag is selected, the data will be divided into n/N groups. If SLIDING window_flag is chosen, the data will be arranged into (n-N) groups, each group starting at one point past the previous. One test result is returned for all groups—if one group fails, FAILED is the result returned; if all groups pass, PASSED is the result returned. In the case of GROUP and n not divisible by N, the last remaining points are ignored. All data selection is done external to multirules. If there is a “missing point” or if “historical data” is required, it is taken care of before the function is called, in the database query, or its equivalent. In the unexpected case of a set of rules having N’s without a common denominator, a group of statistical rules can be used. This can be accomplished by building an object with multiple member statistical rules that returns the result of the cumulative result of its members.

The operation of the sample handling system will now be described in the context of one specific implementation of the invention, it being understood that the invention is not limited to this particular implementation.

Operation of the sample handling system according to the invention is centered on linear track 13 along which cuvettes are advanced from station to station by lead screw 88. The basic timing and sequencing of the system is based on advancing the cuvettes along the linear track a distance equal to the distance between successive reaction wells.

Initially, an operator loads cuvettes into the instrument by placing a cassette of cuvettes into cassette frame 46. Each cassette holds, for example, 120 cuvettes. The cuvettes are automatically moved from the cassette onto linear track 13 by arm 52 where they engage lead screw 88. Each cuvette preferably has four ¼ inch reaction wells. Lead screw 88 is activated every fifteen seconds to move the cuvettes in 0.25 inch increments in 0.1 seconds. The instrument controller monitors each cuvette by the timing associated with the lead screw. Lead screw 88 advances the cuvettes to the first station, i.e., sample insertion station 14, where a sample is delivered to a reaction well aligned with sample probe 36. Two minutes later, the reaction well of the cuvette arrives at the first reagent delivery probe 38 where diluent or a reagent is added, depending on the test being carried out. The second reagent probe 40 is located at the four minute position where an activator can be added. Five minutes later the loaded reaction well of the cuvette reaches the third reagent probe 42 where a reagent is added and the reaction monitoring begins. The reaction is monitored electro-optically by optical monitoring system 22 which measures changes in the optical transmission of the reaction volume as the clot forms or as the chromometric reaction proceeds. As the cuvette is moved along the track, the optical monitoring continues for twenty consecutive stations, that is, for 300 seconds. Following the optical monitoring station the cuvette leaves the track and is sent to a waste container (not shown).

Patient plasma samples are stored in refrigerated housing 26 in the original evacuated blood collection tubes used to obtain the patient’s sample which has been previously spun down to obtain the plasma and bar-coded for patient identification and test protocol to be performed. The evacuated sample collection tubes are placed in the holders of shuttles 28 and advanced by the shuttle drive mechanism to the bar code reader. The evacuated sample collection tubes can be arranged in any order since the bar code on each sample collection tube allows the instrument to automatically correlate a patient with a given sample. The bar code read by bar code reader 32 also programs the instrument controller for determining the amount of sample to be aspirated by sample probe 36, the number of reaction wells to be filled with the sample, and the amounts and types of reagents/buffers/additives/activators to be injected into the respective reaction wells by reagent probes 38, 40 and 42. Subsequent to programming station 30, a sample collection tube is advanced to piercer 34 where piercing tube 152 is caused to pierce the septum of the evacuated sample tube to allow sample probe 36 to be lowered into the sample collection tube to aspirate a programmed amount of sample. Sample probe 36 is next removed from the evacuated sample collection tube and horizontally moved over a reaction well positioned at sample insertion station 14 and lowered into the reaction well where a programmed amount of sample is expelled into the reaction well. The evacuated sample collection tubes can be removed from refrigerated housing 26 at any time after sample aspiration is complete; however, because the samples are maintained at lowered temperatures, they can be retained for further testing without having to be immediately removed from its shuttle. Reagent chamber 35 stores various controls, diluents, activators and reagents. In one implementation of the system up to twenty-two containers of these materials are stored in reagent chamber 35. All containers are held to a temperature of about 7° C. and the reagents are heated, if necessary, in the reagent probe as they are being dispensed.

Pumping in all cases is performed with positive displacement syringe pumps operatively connected with respective ones of the probe. No manipulation of pump tubing is

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required as is the case with peristaltic pumps. A reagent is dispensed into a reaction well in a manner that promotes mixing with the sample and other contents of the reaction well. The reagent temperature and volume are controlled by the instrument controller.

Desirably, fluid level sensing is utilized to control the height of a reagent probe relative to the level of a reagent in its container and relative to the contents of a reaction well. This permits bringing the outside of a probe into contact with a minimum quantity of reagent. This, in turn, reduces the possibility for carry-over. Additionally, level sensing is used to control the height of a probe above the fluid level while dispensing in order to minimize carry-over and to maximize mixing.

At the same time that all of the above mechanical and programmed functions are occurring, various quality assurance/quality control checks are being automatically performed. Also, data calculations are being automatically performed in order to produce and deliver a final result of the parameter being assayed for. This is shown in the following example.

Example for Factor VIII on an Automated Analyzer

A Factor VIII assay was conducted to determine the relative quantity of the intrinsic pathway cofactor VIII (FVIII). FVIII concentration is critical in clot formation and deficiencies of it are symptomatic of a hemophiliac condition. It is important to be able to accurately quantitate the FVIII concentration in patient specimens. The invention addresses all aspects of properly performing the complex FVIII Assay.

A FVIII Assay consists of three types of sample evaluations:

1. Reference materials to normalize the reagent system.
2. Control materials to assure the quality of the reference curve.
3. Patient samples to aid in the diagnosis and treatment of the patient.

Specimen handling and dilutions (Arm 1)

The Factor VIII assay requires the dilution of sample (be it reference, control or patient) serially from a 1:5 to 1:1280. Each type of material requires a different set of dilutions that can be prescribed at time of sampling. The sample was diluted with imidazole buffer that was contained in the ARM1 reservoir. The Factor VIII reference curve was and is typically prepared as follows:

1. 90 uL of Imidazole was loaded into the syringe from the reservoir from arm 1.
2. The arm moved to the appropriate sample reservoir and aspirated 10 uL plasma.
3. The total 100 uL was delivered to the cuvette.
4. The probe then loaded 50 uL of Imidazole from the reservoir for arm 1.
5. 50 uL of the 100 uL was aspirated from the cuvette.
6. The track was incremented forward and the 50 uL aspirated from the previous well along with the 50 uL of Imidazole were delivered into the new well.
7. Steps 4 through 7 were repeated for each additional serial dilution required.

The end result was a FVIII reference curve with seven serial dilutions from a starting dilution of 1:10. The patient and control samples were diluted in the same fashion. Three serial dilutions were performed on a patient sample starting at 1:20, and 2 dilutions on the control material starting at 1:20. The system allows modification of the initial dilution ratio and the number of serial dilutions on a per patient basis.

Quality Checks: The ability to properly perform dilutions was verified using the dye verification process described in

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the sample preparation detailed description (See FIG. 9). If the plasma volume in the primary reservoirs is insufficient, a level sense error is generated. If insufficient volume for the Imidazole reservoir is detected, an error message is given.

5 Reagent Addition and Incubation

After the appropriate volume of plasma and buffer has been added to the test well, then the appropriate reagent must be added at the appropriate times to prepare the well for optical inspection.

10 Addition of factor deficient plasma (Arm 2):

The FVIII assay required the addition of 50 uL of Factor deficient material 2 minutes and 40 seconds after arm 1. The well was still maintained at ambient temperature.

Addition of Activator (Arm 3):

15 The FVIII assay is an APTT based assay (test of the intrinsic pathway) and required the addition of an activator material at arm 3 approximately 2 minutes and 40 seconds after the addition of the factor deficient material. During the 2 minutes and 40 seconds the test well is warmed from ambient temperature to about 33° C., then 50 uL of activator was added at 39° C. raising the temperature in the test well to 37° C. The activator was stored on the reagent tray at 8° C. and mixed automatically to maintain a homogenous suspension.

25 Addition of Calcium Chloride (Arm 4):

The sample was then incubated for 3 minutes 40 seconds at 37° C. The incubation/activation time is critical for clot based test measuring parameters of the intrinsic pathway. At the end of activation, 50 uL of 0.25 M calcium chloride was added at 37° C., starting the reaction.

30 Quality Checks: The correct volumes of reagents delivered was assured by the dye tracking system. The quality of the reagents was assured by using the multirule analysis quality control program defined earlier. The thermal profile was monitored by querying temperature sensors as a test well goes over them. If the temperature is determined to be out of range then an error message is reported to the user.

Optical Inspection

40 The test well was optically inspected for up to 300 seconds. During this inspection process the test well was exposed to fifteen different optical windows for 20 seconds each. A normalization process was required at the first optical windows that normalizes all other windows to the first. Additionally, the normalization process also removes the differences in light levels associated with variability due to the unique turbidity of each plasma. The data was collected for a unique set of assay specific wavelengths and stored in a data file. The data file also contained the data set required to determine quantities of preanalytical variables and dye concentrations for volume verification.

45 Quality Checks: There are quality checks for wavelength verification, optical integrity (Liquid Crystal Clot Simulator), and an acceptable signal to noise ratio as an indication of proper normalization.

55 Data Analysis

Endpoint determinations and calibrations:

All endpoints for the test well data files were computed using the peak of the second derivative described above. The endpoints were standardized using a unique calibration scheme. Systematic bias associated with reagent lot to lot variability and instrument to instrument variability was minimized by calibration of the system using labeled plasma calibrators. For more current coagulation systems, systematic error in the measurement of clotting times is due to instrument thermal and fluidic variation as well as in variation in reagent lot to lot sensitivity. Therefore, reference and therapeutic intervals are specific for each instrument and

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reagent lot and are routinely redetermined. The analyzer's calibration procedure normalized instrument mechanical variables and reagent inter-lot sensitivity, providing uniform APTT clotting times suitable for direct reporting or for use in standardization methods. The analyzer's APTT assays (including FVIII) were calibrated by replicate analysis of Verical Calibration Plasmas™ (Organon Teknika) that have assigned clotting times. Observed values were regressed versus assigned values and the resulting regression coefficients were used to normalize the instrument/reagent system.

Reference Curve Generation

Reference curves were constructed by taking the dilution and known concentration of the reference material and regressing it with the obtained endpoint for each test well. The type of regression and analysis tool used dictates the quality of the reference curve and ultimately the quality of the patient result. The traditional method of constructing a Factor VIII reference curve, log-log transformation, fit to a first order polynomial is not representative of the true biological processes. The curve is better represented by the technique used for this invention. The improvements are most prevalent in the 50 to 100% range and 1 to 10% range, both ranges being clinically significant. The reference curve was constructed by plotting the % activity for the seven dilutions as the independent variable (x), and clotting time as the dependent variable (y). The function is piecewise and discontinuous and composed of two linear sub-curves fit to overlapping sets of data. The first sub-curve uses the first four dilutions and fits a second order polynomial to untransformed data. The second sub-curve fits a second order polynomial to log₁₀ (clot times) and log₁₀ (%activity) to all of the dilutions after the first one. A "cutoff", which determines which sub-curve is used to recover % activity, is established based on the clot time corresponding to the fourth dilution value recovered from the curve. The curve accurately represents the shape of the expected values, and remains robust in the presence of minor variance in the data. FIG. 10 shows a comparison between the traditional Factor VIII reference curve and the automatic analysis Factor VIII assay reference curve.

The patient samples were evaluated with respect to a stored or newly generated reference curve and the reported value was corrected for dilution automatically.

Quality Control Checks: Measures of Goodness of fit for the calibration process and the reference curve structure used were defined earlier. The control values were monitored and subjected to the quality control multirule analysis procedures. The slope of the sample dilutions when compared with the controls or reference curve are determined to assess for the presence of Factor VIII inhibitors such as the Bethesda inhibitor.

It will be understood that the above description of the present invention is susceptible to various modifications, changes and adaptations, and the same are intended to be comprehended within the meaning and range of equivalents of the appended claims.

We claim:

1. A method for performing qualitative and quantitative coagulation, chromogenic and immunological assays for hemostasis and thrombosis parameters on each of a multiplicity of samples of plasma, serum or whole blood, wherein each sample is selected for assay on a random access basis without a drop in speed of sample through-put, comprising:

a) identifying each of a multiplicity of samples, selecting each sample and scheduling one or more of a plurality of assays selected from each of qualitative and quan-

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titative coagulation, chromogenic and immunological assays for hemostasis and thrombosis parameters, to be performed on each sample;

b) providing a plurality of test wells for said plurality of assays and a specimen handling means for automatically transferring at a sample insertion station an aliquot of each sample from a holding device to a respective test well of said plurality of test wells;

c) automatically selecting and adding reagents needed for said plurality of assays selected from each of qualitative and quantitative coagulation, chromogenic and immunological assays to measure hemostasis or thrombosis parameters of the samples in the test wells, wherein in a predetermined first constant time after the sample is delivered to the test well at the sample insertion station, the test well is disposed at a first reagent delivery probe station where a predetermined volume of reagent is optionally added to the test well depending upon the type of assay being performed on the sample in the test well, the volume and temperature of the reagent being variable depending upon which thrombosis or hemostasis assay is being performed;

and wherein in a predetermined second constant time after the test well passes through the first reagent delivery probe station, the test well is disposed at a second reagent delivery probe station where a predetermined volume of a second reagent is optionally added depending upon the type of assay being performed on the sample in the test well, the volume and temperature of the second reagent being variable depending upon which thrombosis or hemostasis assay is being performed;

and wherein in a predetermined third constant time after the test well passes through the second reagent delivery probe station, the test well reaches an optical monitoring station where a reaction of the sample and one or more reagents is monitored by an optical monitoring system for measuring changes in an optical transmission of the sample;

and wherein each of said predetermined constant times remains the same regardless of the hemostasis or thrombosis assay being performed on the sample;

said monitoring of the reaction of the sample comprising detecting the reaction in each test well and measuring data from each reaction,

mathematically processing the measured data to evaluate a change in or magnitude of the measured data from the reaction in the well, and

reporting results of the processing of the measured data.

2. The method according to claim 1, further comprising independently defining a test protocol for each assay.

3. The method according to claim 2, wherein the test protocol includes mechanical instructions, optical instructions, data analysis and quality assurance parameters.

4. The method according to claim 1, further comprising automatically diluting the transferred sample.

5. The method according to claim 1, further comprising:

a) controlling a cooling unit and maintaining each sample initially within a low temperature range having a predetermined maximum temperature;

b) controlling a heating unit that maintains each sample within a predetermined measuring temperature range that is higher than the initial temperature range;

c) transitioning the temperature of each sample from the initial range to the measuring temperature;

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d) reducing an effect of ambient temperature on each sample; and

e) delivering reagents to each test well at a specified temperature.

6. The method according to claim 1, wherein after dispensing a sample or reagent into a well, a sample preparation means for automatically selecting and adding the reagents for the scheduled assays is washed by a wash liquid.

7. The method according to claim 6, wherein the wash liquid is selected from a group consisting of water, wash solution and bleach solution.

8. The method according to claim 1, further comprising evaluating data obtained from each assay by

a) calculating a second derivative of a process signal and determining the peak thereof, which is an indication of maximum acceleration of clot formation; or

b) calculating a magnitude of change of a process signal; or

c) calculating a rate of change of a parameter, whereby a first derivative of the parameter is determined; or

d) a combination of the calculations of a)–c) above.

9. The method according to claim 1, wherein the data is transformed by normalization and calibration to standardized material by a numerical construction of a system model using known reference material, specific for each assay.

10. The method according to claim 9, further comprising a system model developed from a function with a linear or nonlinear relationship between recovered and reported values, the function created by fitting to a set of sample data.

11. The method according to claim 1, comprising a quality assurance protocol for monitoring and evaluating the sample handling, sample preparation, reaction detection and results reported for the sample, wherein the quality assurance protocol comprises checks for sample integrity, reagent integrity, mechanical suitability and optical quality.

12. The method according to claim 11, wherein the check for sample integrity comprises detecting at least one pre-analytical variable in the sample selected from lipemia, bilirubin and hemolysis.

13. The method according to claim 11, wherein the check for reagent integrity comprises evaluating control material and applying statistical quality control rules and a comparison of a current result to an earlier sample result.

14. The method according to claim 11, wherein the check for system suitability comprises measuring electrical, volumetric and thermal output of critical mechanical components.

15. The method according to claim 11, wherein the check for optical quality comprises monitoring an optical reference clot at an appropriate wavelength and monitoring the signal to noise ratio, thereby insuring the use of acceptable signals.

16. An instrument for automatically performing qualitative and quantitative coagulation, chromogenic and immunological assays for hemostasis and thrombosis parameters on each of a multiplicity of samples of plasma, serum or whole blood, whereby each sample is selected for assay on a random access basis without a drop in speed of sample through-put, comprising:

a) a programming input device for identifying each of a multiplicity of samples, and means for selecting each sample and scheduling one or more hemostasis and thrombosis assays to be performed on each sample on a random access basis;

b) a plurality of test wells for a plurality of assays and a specimen handling means for automatically transferring at a sample insertion station an aliquot of each

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sample from a holding device to a respective test well of said plurality of test wells;

c) a fully automated sample preparation means for automatically selecting and adding reagents needed for a plurality of qualitative and quantitative coagulation, chromogenic and immunological assays to measure hemostasis or thrombosis parameters of the samples in the test wells, a first reagent delivery probe station being provided where a diluent or reagent is optionally added to a test well depending upon the type of assay being performed on the sample in the test well, the test well being disposed at said first reagent delivery probe station in a predetermined first constant time after the sample is delivered to the test well at said sample insertion station, said sample preparation means comprising means for varying at said first reagent delivery probe station the type of reagent and the volume and temperature of the reagent depending upon which thrombosis or hemostasis assay is being performed;

a second reagent delivery probe station being provided where a reagent is optionally added depending upon the type of assay being performed on the particular sample in the test well, the test well being disposed at said second reagent delivery probe station in a predetermined second constant time after the test well passes through said first reagent delivery probe station, said sample preparation means comprising a means for varying at said second reagent delivery probe station the type of reagent and the volume and temperature of the reagent depending upon which thrombosis or hemostasis assay is being performed;

an optical monitoring station being provided where a reaction of the sample and one or more reagents is monitored by an optical monitoring system for measuring changes in the optical transmission of the sample, said test well reaching said optical monitoring station in a predetermined third constant time after the test well passes through said second reagent delivery probe station, and wherein each of said predetermined constant times remains the same regardless of the hemostasis or thrombosis assay being performed on the sample;

said optical monitoring system comprising a detector for detecting the reaction in each test well and measuring the data from each reaction,

a processor for mathematically processing the measured data to evaluate a change in or magnitude of the measured data from the reaction in the well, and

a reporter to report results of processing the measured data by the processor.

17. The instrument according to claim 16, wherein additionally is included a means for independently defining required steps for each assay.

18. The instrument according to claim 19, wherein instructions for a scheduled assay include mechanical instructions, optical instructions, data analysis and quality assurance parameters.

19. The instrument according to claim 16, wherein additionally is included a diluting device for automatically diluting the transferred sample.

20. The instrument according to claim 16, further comprising:

a) a cooling controller for independently controlling a cooling unit, maintaining each sample initially within a low temperature range having a predetermined maximum temperature;

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- b) a heating controller for independently controlling a heating unit that maintains each sample in an optically monitored section within the detector, within a predetermined measuring temperature range that is higher than the initial temperature range;
- c) a transitioning device for transitioning each sample from the initial range to the measuring temperature;
- d) a reducing device for reducing the effect of ambient temperature on each sample; and
- e) a transport mechanism for delivering reagents to the test well at a specified temperature.

21. The instrument according to claim 16, further comprising a washing mechanism wherein following each time a sample or reagent is dispensed into a well, the sample preparation means is washed by the washing mechanism with a wash liquid.

22. The instrument according to claim 21, wherein the wash liquid is selected from a group consisting of water, wash solution or bleach solutions.

23. The instrument according to claim 16, further comprising a computational means for evaluating data obtained from each assay, the computational means comprising:

- a) means for calculating a second derivative of a process signal and determining the peak thereof, which is an indication of maximum acceleration of clot formation; or
- b) means for calculating a magnitude of change of a process signal; or
- c) means for calculating a rate of change of a parameter, whereby a first derivative of the parameter is determined; or
- d) a combination of the means for calculating of a)-c) above.

24. The instrument according to claim 16, wherein the change in or magnitude of the data is measured by a means for normalization and calibration to standardized material by a numerical construction of a system model using known reference material, specific for each assay.

25. The instrument according to claim 24, further comprising a system model developed from a function with a linear relationship between recovered and reported values, the function created by fitting to a set of sample data.

26. The instrument according to claim 16, wherein a quality assurance means is provided comprising a means for checking for sample integrity, reagent integrity, mechanical suitability and optical quality.

27. The instrument according to claim 26, wherein the means for checking for sample integrity comprises detecting preanalytical variables in the sample.

28. The instrument according to claim 26, wherein the means for checking for reagent integrity comprises evaluating control material and applying statistical quality control rules and a comparison of a current result to a earlier sample result.

29. The instrument according to claim 26, wherein the means for checking for mechanical suitability comprises a means for measuring electrical, volumetric and thermal output of mechanical components of said instrument.

30. The instrument according to claim 26, wherein the means for checking for optical quality comprises a means for monitoring an optical reference clot at an appropriate wavelength inspection and monitoring a signal to noise ratio.

31. The method of claim 1, wherein a plurality of cuvettes are provided, each comprising a plurality of said test wells, and wherein different assays selected from said qualitative

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and quantitative coagulation, chromogenic and immunological assays for measuring hemostasis or thrombosis parameters are performed on one or more samples in different test wells of the same cuvette.

32. The method of claim 1, wherein said different assays comprise Partial Thromboplastin Time, Prothrombin Time, Activated Partial Thromboplastin Time and Factor deficiency assays.

33. The method of claim 1, wherein said first and second predetermined constant times are about two minutes and forty seconds.

34. A method for performing assays to measure hemostasis or thrombosis parameters, comprising:

- a) identifying each of a plurality of samples, selecting each sample and scheduling one or more of hemostasis and thrombosis assays to be performed on each sample;
- b) providing a plurality of test wells for a plurality of assays and a specimen handler for automatically transferring at a sample insertion station an aliquot of each sample from a holding device to a respective test well of said plurality of test wells;
- c) automatically selecting and adding reagents needed for a plurality of different scheduled assays with a sample preparation device to measure hemostasis or thrombosis parameters of the samples in the test wells, wherein in a predetermined first constant time after the sample is delivered to the test well at the sample insertion station, the test well is disposed at a first reagent delivery probe station where a predetermined volume of reagent is optionally added to the test well depending upon the type of assay being performed on the sample in the test well, the volume and temperature of the reagent being variable depending upon which thrombosis or hemostasis assay is being performed;

and wherein in a predetermined second constant time after the test well passes through the first reagent delivery probe station, the test well is disposed at a second reagent delivery probe station where a predetermined volume of a second reagent is optionally added depending upon the type of assay being performed on the sample in the test well, the volume and temperature of the second reagent being variable depending upon which thrombosis or hemostasis assay is being performed;

and wherein in a predetermined third constant time after the test well passes through the second reagent delivery probe station, the test well reaches an optical monitoring station where a reaction of the sample and one or more reagents is monitored by an optical monitoring system for measuring changes in an optical transmission of the sample;

and wherein each of said predetermined constant times remains the same regardless of the hemostasis or thrombosis assay being performed on the sample; and wherein said plurality of test wells for said plurality of assays are moved in the same path relative to said sample preparation means irrespective of the assay being performed on each test well, and wherein a temperature at which said path is maintained and the rate at which the test wells are moved along the path does not vary from assay to assay such that different hemostasis and thrombosis assays can be randomly, continuously and substantially concurrently performed on a fully automated, random access basis;

said monitoring of the reaction of the sample comprising detecting the reaction in each test well and measuring data from each reaction,

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mathematically processing the measured data to evaluate a change in or magnitude of the measured data from the reaction in the well, and

reporting results of the processing of the measured data.

35. The method of claim 34, wherein a plurality of 5
cuvettes are provided, each comprising a plurality of said test wells, and wherein said different assays for measuring thrombosis or hemostasis parameters are performed on one or more samples in different test wells of the same cuvette.

36. The method of claim 34, wherein said different assays 10
comprise Partial Thromboplastin Time, Prothrombin Time, Activated Partial Thromboplastin Time and Factor deficiency assays.

37. The method of claim 34, wherein said first and second 15
predetermined constant times are about two minutes and forty seconds.

38. An instrument for automatically performing assays to measure hemostasis or thrombosis parameters, comprising:

a) a programming input device for identifying each of a 20
plurality of samples and selecting each sample and scheduling one or more of hemostasis and thrombosis assays to be performed on each sample;

b) a plurality of test wells for a plurality of assays and a 25
specimen handler for automatically transferring at a sample insertion station an aliquot of each sample from a holding device to a respective test well of said plurality of test wells;

c) a sample preparation device for automatically selecting 30
and adding reagents needed for a plurality of different scheduled assays to measure hemostasis or thrombosis parameters of the samples in the test wells, a first reagent delivery probe station being provided where a diluent or reagent is optionally added to a test well depending upon the type of assay being performed on 35
the sample in the test well, the test well being disposed at said first reagent delivery probe station in a predetermined first constant time after the sample is delivered to the test well at said sample insertion station, said sample preparation means comprising means for 40
varying at said first reagent delivery probe station the type of reagent and the volume and temperature of the reagent depending upon Which thrombosis or hemostasis assay is being performed;

a second reagent delivery probe station being provided 45
where a reagent is optionally added depending upon the type of assay being performed on the sample in the test well, the test well being disposed at said second reagent delivery probe station in a predetermined second con-

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stant time after the test well passes through said first reagent delivery probe station, said sample preparation means comprising a means for varying at said second reagent delivery probe station the type of reagent and the volume and temperature of the reagent depending upon which thrombosis or hemostasis assay is being performed;

an optical monitoring station being provided where a reaction of the sample and one or more reagents is monitored by an optical monitoring system for measuring changes in an optical transmission of the sample, said test well reaching said optical monitoring station in a predetermined third constant time after the test well passes through said second reagent delivery probe station, and wherein each of said predetermined constant times remains the same regardless of the hemostasis or thrombosis assay being performed on the sample, and wherein a temperature at which said path is maintained is constant from assay to assay and the rate at which the test wells are moved along the path by said mover does not vary from assay to assay such that different hemostasis and thrombosis assays can be randomly, continuously and substantially concurrently performed on a fully automated, random access basis; said optical monitoring system comprising a detector for detecting the reaction in each test well and measuring data from each reaction,

a processor for mathematically processing the measured data to evaluate a change in or magnitude of the measured data from the reaction in the well, and reporting results of processing the measured data.

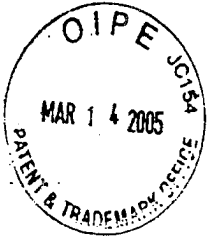
39. The method of claim 1, wherein prior to said test well reaching said optical monitoring system, said test well passes through a third reagent delivery probe station where a reagent is optionally added depending upon the type of assay being performed on the particular sample in the test well, the type of reagent and the volume and temperature of the reagent being variable depending upon which thrombosis or hemostasis assay is being performed.

40. The method of claim 34, wherein prior to said test well reaching said optical monitoring system, said test well passes through a third reagent delivery probe station where a reagent is optionally added depending upon the type of assay being performed on the particular sample in the test well, the type of reagent and the volume and temperature of the reagent being variable depending upon which thrombosis or hemostasis assay is being performed.

* * * * *

EXHIBIT

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 97,008-X1)

In re Application of:)	
)	
COPELAND, et. al)	
)	Group Art Unit: 1743
Serial No.: 10/991,050)	
)	Examiner: Brian Gordon
Filed: November 17, 2004)	
)	
For: Automated Biological)	
Reaction Apparatus)	

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SECOND PRELIMINARY AMENDMENT

Dear Sir:

This is a Second Preliminary Amendment for the above-identified patent application.

Amendments to the Claims are reflected in the **Listing of the Claims** which begins on page 2 of this paper.

Remarks begin on page 12 of this paper.

IN THE CLAIMS:

This listing of claims will replace all prior versions, and listing, of claims in the application.

Listing Of The Claims:

1-71 (Cancelled)

72. (Previously presented) An automated method of dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container;

providing at least one slide on a slide support;

automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container;

automatically determining whether reagent from the reagent container should be dispensed onto the slide; and

automatically dispensing the reagent onto the slide based on the determination of whether the reagent should be dispensed onto the slide, wherein the step of automatically determining whether reagent should be dispensed onto the slide includes acquiring information from an optically-encoded identifier associated with the slide.

73. (Previously presented) The automated method of claim 72 wherein said information associated with the reagent container comprises at least one optically-encoded identifier.

74. (Currently amended) The automated method of claim 73 wherein said at least one reagent container optically-encoded identifier ~~comprises~~ is selected from the group consisting of a bar code, text, and combinations thereof.

75. (Currently amended) The automated method of claim 72 wherein said optically-encoded identifier associated with the slide comprises is selected from the group consisting of a bar code, text, and combinations thereof.

76. (Previously presented) The automated method of claim 73 wherein the step of automatically identifying the reagent container comprises reading the reagent container optically-encoded identifier to identify the reagent.

77. (Previously presented) The automated method of claim 76 wherein the reagent container optically-encoded identifier comprises a bar code.

78. (Previously presented) The automated method of claim 72 wherein the step of automatically determining whether reagent from the reagent container should be dispensed onto the slide comprises using an optically-encoded identifier to identify a staining protocol to be applied to the slide.

79. (Previously presented) The automated method of claim 78 wherein said optically-encoded identifier comprises a bar code.

80. (Previously presented) The method of claim 78 wherein said staining protocol information is located remote from said optically-encoded identifier.

81. (Previously presented) A method of automatically dispensing reagents onto a slide, the method comprising the steps of:

providing at least one reagent container containing a reagent, said reagent container also having optically-encoded information associated with it;

providing at least one slide on a slide support, said slide having optically-encoded information associated with it;

automatically identifying said reagent container using a computer, the step of automatically identifying including reading said reagent container optically-encoded information; and

automatically determining whether said reagent from said reagent container should be dispensed onto said slide based on reading said optically-encoded information associated with said slide.

82. (Previously presented) The automated method of claim 81 wherein said optically-encoded information associated with the reagent container comprises at least one optical identifier.

83. (Currently amended) The automated method of claim 82 wherein said at least one optical identifier ~~comprises~~ is selected from the group consisting of bar code-encoded information and textual information.

84. (Previously presented) The automated method of claim 81 wherein said optically-encoded information from the at least one slide comprises at least one optical identifier.

85. (Previously presented) The automated method of claim 82 wherein the step of automatically identifying the at least one reagent container comprises reading the optical identifier associated with the reagent container to identify the reagent contained in the reagent container.

86. (Currently amended) The automated method of claim 85 wherein the optical identifier associated with the reagent container ~~comprises~~ is selected from the group consisting of bar code-encoded information and textual information.

87. (Previously presented) The automated method of claim 81 wherein the step of automatically determining whether reagent from the at least one reagent container should be dispensed onto the at least one slide comprises using an optical identifier to identify a staining protocol to be applied to the slide.

88. (Currently amended) The automated method of claim 87 wherein said optical identifier used to identify a staining protocol comprises bar code-encoded information.

89. (Previously presented) The method of claim 88 wherein said staining protocol information is located remote from said optical identifier.

90. (Previously presented) An automated method of dispensing reagents onto a slide, the method comprising the steps of:

- providing at least one reagent container;
- providing at least one slide on a slide support;
- automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container;
- automatically determining whether the reagent from the reagent container should be dispensed onto the slide; and

- automatically dispensing the reagent onto the slide based on the determination of whether the reagent should be dispensed onto the slide, wherein the step of automatically determining whether reagent should be dispensed onto the slide includes acquiring machine-readable information associated with the slide.

91. (Previously presented) The automated method of claim 90 wherein said information associated with the reagent container comprises at least one machine-readable identifier.

92. (Previously presented) The automated method of claim 91 wherein said at least one machine-readable identifier comprises an optical identifier.

93. (Currently amended) The automated method of claim 92 wherein said optical identifier ~~comprises~~ is selected from the group consisting of a bar code, text, and combinations thereof.

94. (Previously presented) The automated method of claim 91 wherein the step of automatically identifying the reagent container comprises reading the machine-readable identifier associated with the reagent container to identify the reagent.

95. (Previously presented) The automated method of claim 94 wherein the machine-readable identifier associated with the reagent container comprises optically-encoded information.

96. (Previously presented) The automated method of claim 90 wherein the step of automatically determining whether reagent from the reagent container should be dispensed onto the slide comprises using a machine-readable identifier to identify a staining protocol to be applied to the slide.

97. (Previously presented) The automated method of claim 96 wherein said machine-readable identifier comprises optically-encoded information.

98. (Previously presented) The method of claim 96 wherein said staining protocol information is located remote from said machine-readable identifier.

99. (Previously presented) The method of claim 98 wherein said machine-readable identifier comprises optically-encoded information.

100. (Previously presented) An automated method of dispensing reagents onto a slide, the method comprising the steps of:

- providing at least one reagent container;
- providing at least one slide on a slide support;
- automatically identifying the reagent container using a computer, the step of automatically identifying being based on information associated with the reagent container;
- automatically determining whether reagent in the reagent container should be dispensed onto the slide; and
- automatically dispensing the reagent onto the slide based on the determination of whether the reagent should be dispensed onto the slide, wherein the step of automatically determining whether reagent should be dispensed onto the slide includes acquiring information from an optically-encoded symbol associated with the slide.

101. (Previously presented) The automated method of claim 100 wherein said information associated with the reagent container comprises at least one optically-encoded symbol.

102. (Previously presented) The automated method of claim 101 wherein said reagent container-associated optically-encoded symbol comprises a bar code.

103. (Previously presented) The automated method of claim 101 wherein the step of automatically identifying the reagent container comprises reading the optically-encoded symbol associated with the reagent container to identify the reagent.

104. (Previously presented) The automated method of claim 103 wherein the optically-encoded symbol comprises a bar code.

105. (Previously presented) The automated method of claim 100 wherein the step of automatically determining whether reagent from the reagent container should be dispensed onto the slide comprises using an optically-encoded symbol to identify a staining protocol to be applied to the slide.

106. (Previously presented) The automated method of claim 105 wherein said optically-encoded symbol comprises a bar code.

107. (Previously presented) The method of claim 105 wherein said staining protocol information is located remote from said optically-encoded symbol.

108. (New) The automated method of claim 76 wherein the reagent container optically-encoded identifier is text.

109. (New) The automated method of claim 78 wherein said optically-encoded identifier is text.

110. (New) The automated method of claim 82 wherein said at least one optical identifier is bar code-encoded information.

111. (New) The automated method of claim 82 wherein said at least one optical identifier is textual information.

112. (New) The automated method of claim 85 wherein the optical identifier associated with the reagent container is bar code-encoded information.

113. (New) The automated method of claim 85 wherein the optical identifier associated with the reagent container is textual information.

114. (New) The automated method of claim 92 wherein said optical identifier is a bar code.

115. (New) The automated method of claim 92 wherein said optical identifier is text.

116. (New) The automated method of claim 101 wherein said reagent container-associated optically-encoded symbol comprises text.

117. (New) The automated method of claim 103 wherein the optically-encoded symbol comprises text.

118. (New) The automated method of claim 105 wherein said optically-encoded symbol comprises text.

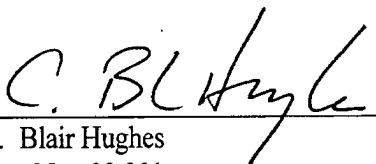
REMARKS

Claims 72-118 are pending in the application. New claims 108-118 have been added to the application. Claims 74-75, 83, 86, and 93 are amended to more clearly claim what it is that the Applicant's regard as their invention. Specifically, the applicant's have included text as an optical identifier in the claims. No new matter has been added to the application by way of these claim amendments.

Respectfully submitted,

McDonnell Boehnen Hulbert & Berghoff

Date: March 8, 2005

By: 
A. Blair Hughes
Reg. No. 32,901
312-913-2123

EXHIBIT

22

**UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS**

8/5

VENTANA MEDICAL SYSTEMS, INC.,

Plaintiff,

v.

VISION BIOSYSTEMS INC.,

Defendant.

Civil Action No. 05-CV-10614-GAO

**VENTANA'S RESPONSES TO VISION'S FIRST SET
OF INTERROGATORIES, NOS. 1 TO 12**

Plaintiff Ventana Medical Systems, Inc. ("Ventana") objects and responds to Defendant Vision BioSystems, Inc.'s First Set of Interrogatories, Nos. 1 to 12, as follows.

GENERAL OBJECTIONS

1. Ventana objects to the interrogatories as unduly burdensome and oppressive to the extent they purport to require Ventana to search Ventana facilities and inquire of Ventana employees other than those facilities and employees that would reasonably be expected to have responsive information. To the extent that Ventana produces information, it will do so based upon (1) a reasonable search, given the time allotted to Ventana to respond to the interrogatories, of facilities and files that could reasonably be expected to have responsive information, and (2) inquiries of Ventana employees and/or representatives who could reasonably be expected to have responsive information.

2. Ventana objects to the interrogatories to the extent they seek to impose any requirement on Ventana other than those set forth in the Federal Rules of Civil Procedure.

3. Ventana objects to the interrogatories to the extent they seek information protected by the attorney-client privilege, the attorney work product doctrine or any other

applicable immunity, privilege, protection or rule of confidentiality that makes information non-discoverable. Such information will not be produced. Any inadvertent disclosure of such information shall not be deemed a waiver of the attorney-client privilege, the attorney work product doctrine or any other applicable privilege or immunity recognized by statute or case law.

4. The parties have agreed that information produced in *Vision BioSystems (USA) Trading Inc., v. Ventana Medical Systems, Inc.*, Civil Action No. 03-CV-10391-GAO ("*Vision I*") shall be deemed produced in the present case ("*Vision II*"), with each party reserving all objections. Therefore, Ventana objects to the interrogatories to the extent they request information already produced to Vision in *Vision I*.

5. Ventana objects to the interrogatories to the extent they purport to require Ventana to provide trade secret or other confidential research, development, or commercial information. Ventana will provide such information pursuant to the parties' agreement that the Stipulated Protective Order entered in *Vision I* shall control the exchange of confidential information in *Vision II*.

6. Ventana objects to the interrogatories to the extent that they purport to require Ventana to disclose information in violation of a legal or contractual obligation of nondisclosure to a third party. Ventana will not produce such information without either the consent of the relevant third party, a court order modifying the relevant protective order, or a court order compelling disclosure.

7. Ventana objects to the interrogatories to the extent they seek information shielded from disclosure by any applicable privacy laws.

8. Ventana objects to the interrogatories to the extent they seek information not relevant to the claims or defenses of any party, or not reasonably calculated to lead to the discovery of admissible evidence.

9. Ventana objects to producing and/or identifying on a privilege log any information created or generated in connection with legal representation provided to Ventana in this lawsuit or in connection with the matter of *Vision I*.

10. Ventana objects to the interrogatories to the extent that they seek information already in Vision's possession, custody or control, or available to Vision from public sources.

11. The Court has consolidated *Vision I* and *Vision II* and set the consolidated case for an expedited trial on the merits and ruled that discovery related to the validity of the '861 patent is completed. Given the current trial date, the parties agreed that the disposition of plaintiff's motion for preliminary injunction is currently unnecessary, and that discovery directed to issues uniquely related to that motion is not necessary or appropriate. The parties have also agreed to bifurcate the issues of damages and willfulness. Therefore, Ventana objects to the interrogatories to the extent they seek information related to the validity of the '861 patent, issues uniquely related to Ventana's preliminary injunction motion (such as irreparable harm or balancing of interests), damages or willfulness. Pursuant to this Court's ruling on July 20, 2005, Ventana will respond to the interrogatories only to the extent they seek discovery on the issue of whether Vision's Bond – OCR infringes the '861 patent.

12. In providing responses to the interrogatories, Ventana does not waive but rather preserves: (a) the right to object on any basis permitted by law to the use of any such information, for any purpose, in whole or in part, in any subsequent proceeding in this action or any other action; (b) the right to object on any basis permitted by law to any other discovery request or proceeding involving or relating to the subject matter of these responses; and (c) any and all privileges and/or rights under the Federal Rules of Civil Procedure, other applicable statutes, or common law.

13. Ventana objects to the definitions of "Ventana," "Plaintiff," "You," and "Your" as vague, overly broad, unduly burdensome, oppressive, seeking information outside the scope of permissible discovery, information not in the possession, custody or control of Ventana and information from or about third parties that may be subject to nondisclosure agreements or

other conditions. Ventana further objects to the definitions to the extent they seek information that is protected by the attorney-client privilege and/or the attorney work product doctrine.

14. Ventana objects to the definitions of "Vision" and "Defendant" as vague, overly broad, unduly burdensome, oppressive, seeking information outside the scope of permissible discovery, information not in the possession, custody or control of Ventana and information from or about third parties that may be subject to nondisclosure agreements or other conditions. Ventana objects to the definitions of "Vision" and "Defendant" and the language "all predecessors, subsidiaries, parents, and affiliates (including, without limitation, Vision BioSystems, Ltd. and Vision Systems, Ltd.) and all past or present directors, officers, agents, representatives, employees, entities acting in joint-venture or partnership relationships with Vision, and others acting on behalf of Vision" as vague, overly broad, unduly burdensome, and oppressive to the extent they purport to require Ventana to draw legal conclusions or otherwise speculate as to the identity of persons or entities falling within the scope of these definitions. Ventana interprets "Vision" and "Defendant" to mean Vision BioSystems, Inc.

15. Ventana objects to the definitions of "Process" and "Sub-Process" as vague, overbroad, unduly burdensome and seeking information not relevant to the claims or defenses of any party.

16. Ventana objects to the definition of "Slide Processing System" as vague, overbroad, unduly burdensome and seeking information not relevant to the claims or defenses of any party. Ventana will only produce information relevant to its claim that the Bond – OCR infringes the '861 patent.

17. Ventana objects to the definitions of "and" and "or" as vague, ambiguous, overbroad and unduly burdensome. Ventana shall construe "and" and "or" by their plain meaning, and shall not understand these two words to be interchangeable.

18. Ventana objects to Instructions 1-2 to the extent they create compound interrogatories.

19. Ventana objects to Instructions 1-5 to the extent they seek to impose any requirement on Ventana other than those set forth in the Federal Rules of Civil Procedure.

SPECIFIC OBJECTIONS AND RESPONSES

Ventana expressly incorporates the above general objections as though fully set forth in response to each of the following individual interrogatories. Any response to an interrogatory shall not be deemed a waiver of any applicable specific or general objections to an interrogatory.

INTERROGATORY NO. 1:

Explain the bases for the statement, set forth in Paragraph 6 of the Complaint that "Vision is infringing the '861 patent by making, using, offering to sell and/or selling an apparatus known as the Bond-OCR."

Your reply should identify the claims of the patents-in-suit that you believe will be infringed by the Bond™-OCR instrument (hereinafter "asserted claims"), and should include, for each asserted claim, a claim chart showing your purported construction of each asserted claim limitation and showing one to one correspondence between the asserted claim limitations and the alleged corresponding structure of the Bond™-OCR instrument, including an indication as to whether the correspondence shown is literal or under the doctrine of equivalents.

RESPONSE TO INTERROGATORY NO. 1:

Ventana objects to this interrogatory as duplicative, overbroad, unduly burdensome, and oppressive to the extent that it calls for information that is publicly available or already in Vision's possession or control in connection with *Vision I*.

Ventana further objects to this interrogatory to the extent it calls for information that is protected by the attorney-client privilege and/or the attorney work product doctrine.

Ventana further objects to this interrogatory and the language "Your reply should identify the claims of the patents-in-suit that you believe will be infringed by the Bond™-OCR instrument (hereinafter "asserted claims"), and should include, for each asserted claim, a claim chart showing your purported construction of each asserted claim limitation and showing one to

one correspondence between the asserted claim limitations and the alleged corresponding structure of the Bond™-OCR instrument, including an indication as to whether the correspondence shown is literal or under the doctrine of equivalents” as compound.

Subject to the foregoing objections and its General Objections, Ventana asserts that Vision directly infringes, contributes to the infringement of, and induces infringement of claims 1, 2, 3, 5, 6 and 8 of the ‘861 patent by making, using, offering to sell, selling and/or importing the Bond-OCR instrument, and activities related thereto, including without limitation installation of components of the Bond-OCR instrument into Bond instruments that were found to infringe as a matter of law in *Vision I*. Ventana has provided a detailed element by element analysis of its assertion of infringement in Appendix A to Ventana Medical Systems, Inc.’s Memorandum in Support of Motion for Preliminary Injunction. Ventana further refers Vision to its claim chart evaluating infringement by the Bond-OCR instrument, also attached to Appendix A to Ventana Medical Systems, Inc.’s Memorandum in Support of Motion for Preliminary Injunction. Other than Vision’s substitution of stylized numeric symbols for one-dimensional bar code symbols on slides in the Bond-OCR instrument, Ventana believes that the Bond-OCR instrument is essentially identical to the Bond instruments that were the subject of *Vision I*. Ventana further responds that the Bond-OCR infringes the asserted claims of the ‘861 patent literally or, in the alternative, under the doctrine of equivalents.

Fact discovery is on-going, including discovery of Vision related to the structure, function and operation of the Bond-OCR. Further, infringement by the Bond-OCR will be the subject of expert discovery. Therefore, Ventana reserves the right to supplement the answer to this interrogatory as additional information is developed or becomes available and with any expert reports it submits on infringement by the Bond-OCR.

INTERROGATORY NO. 2:

Identify every patent and patent application in the United States or any foreign country which is based on a specification substantially the same as that for the patent-in-suit, makes any claim to priority based on the filing date of the application for the patent-in-suit, or from which

CERTIFICATE OF SERVICE

I hereby certify that a true and correct copy of the foregoing pleading was served on counsel for defendants in this matter on this 15th day of August, 2005 as follows:

VIA E-MAIL

Elizabeth A. Leff, Esq.
Rothwell, Figg, Ernst & Manbeck, P.C.
1425 K Street, NW, Suite 800
Washington, DC 20005

VIA HAND DELIVERY

Christine M. Roach, Esq.
Roach & Carpenter, PC
24 School Street
Boston, MA 02108



Brian L. Michaelis

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